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METHOD DEVELOPMENT AND VALIDATION BY RP-HPLC FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND VILDAGLIPTINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTERACT

Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages. In present experimentation the standard preparations were made from the API and sample preparations are from formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The average percentage assay was calculated and found to be 99.87% and 100.16 % for Metformin and Vildagliptin respectively. A simple, accurate, precise method was developed for the simultaneous estimation of the Metformin and Vildagliptin in Tablet dosage form. Retention time of Metformin and Vildagliptin were found to be 2.8min and 4.0min. % RSD of the Metformin and Vildagliptin were and found to be 0.65 and 0.9 respectively. % Recover was Obtained as 99.83% and 99.97% for Metformin and Vildagliptin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Vildagliptin were 0.4ppm, 1.3ppm and 0.8ppm, 2.5ppm respectively. Regression equation of Metformin is y = 13779x + 1840, and of Vildagliptin is y = 16828x + 4143.

KEYWORDS: Validation, API, homogeneous, simultaneous estimation, LOD, LOQ etc.

INTRODUCTION

Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages. In the pharmaceutical industry, it is very important that in addition to final testing and compliance of products, it is also assured that the process will consistently produce the expected result. The desired results are established in terms of specifications for outcome of the process. Qualification of systems and equipment is therefore a part of the process of validation.[1] Validation is a requirement of food, drug and pharmaceutical regulating agencies such as the US FDA and their good manufacturing practices guidelines. Since a wide variety of procedures, processes, and activities need to be validated, the field of validation is divided into a number of subsections including the following.[2-4]

A. Equipment validation.
B. Facilities validation.
C. HVAC system validation.
D. Cleaning validation
E. Process Validation.
F. Analytical method validation.
G. Computer system validation.
H. Packaging validation.
I. Cold chain validation.

Similarly, the activity of qualifying systems and equipment is divided into a number of subsections including the following:

A. Design qualification (DQ).
B. Component qualification (CQ).
C. Installation qualification (IQ).
D. Operational qualification (OQ).
E. Performance qualification (PQ).

Reasons for validation

FDA, or any other food and drugs regulatory agency around the globe not only ask for a product that meets its specification but also require a process, procedures, intermediate stages of inspections, and testing adopted during manufacturing are designed such that when they are adopted they produce consistently similar, reproducible, desired results which meet the quality standard of product being manufactured, such procedures are developed through the process of validation. This is

to maintain and assure a higher degree of quality of food and drug products. Validation is "Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.[5] A properly designed system will provide a high degree of assurance that every step, process, and change has been properly evaluated before its implementation. Testing a sample of a final product is not considered sufficient evidence that every product within a batch meets the required specification.

Computer System Validation

This requirement has naturally expanded to encompass computer systems used both in the development and production of, and as a part of pharmaceutical products, medical devices, food, blood establishments, tissue establishments, and clinical trials. In 1983 the FDA published a guide to the inspection of Computerized Systems in Pharmaceutical Processing, also known as the 'bluebook'.[6] Recently both the American FDA and the UK Medicines and Healthcare products Regulatory Agency have added sections to the regulations specifically for the use of computer systems. In the UK, computer validation is covered in Annex 11 of the EU GMP regulations (EMEA 2011). The FDA introduced 21 CFR Part 11 for rules on the use of electronic records, electronic signatures (FDA 1997). The FDA regulation is harmonized with ISO 8402:1994[7] which treats "verification" and "validation" as separate and distinct terms. On the other hand, many software engineering journal articles and textbooks use the terms "verification" and "validation" interchangeably, or in some cases refer to software "verification, validation, and testing (VV&T)" as if it is a single concept, with no distinction among the three terms. The General Principles of Software Validation (FDA 2002) defines verification as "Software verification provides objective evidence that the design outputs of a particular phase of the software development life cycle meet all of the specified requirements for that phase".[8] It also defines Validation as "Confirmation by examination and provision of objective evidence that software specifications conform to user needs and intended uses, and that the particular requirements implemented through software can be consistently fulfilled". The software validation guideline states: “The software development process should be sufficiently well planned, controlled, and documented to detect and correct unexpected results from software changes." Annex 11 states "The validation documentation and reports should cover the relevant steps of the life cycle."

Weichel (2004) recently found that over twenty warning letters issued by the FDA to pharmaceutical companies specifically cited problems in Computer System Validation between 1997 and 2001.[9]

DRUG PROFILE

Metformin: Metformin marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes,[10, 11] particularly in people who are overweight.[12] It is also used in the treatment of polycystic ovary syndrome.[3] Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes.[13, 14] It is not associated with weight gain.[14] It is taken by mouth. Metformin is generally well tolerated.[15] Common side effects include diarrhea, nausea and abdominal pain. It has a low risk of causing low blood sugar.[10] High blood lactic acid level is a concern if the drug is prescribed inappropriately and in overly large doses.[16] It should not be used in those with significant liver disease or kidney problems.[10] While no clear harm comes from use during pregnancy, insulin is generally preferred for gestational diabetes. Metformin is in the biguanide class. It works by decreasing glucose production by the liver and increasing the insulin sensitivity of body tissues.[10]

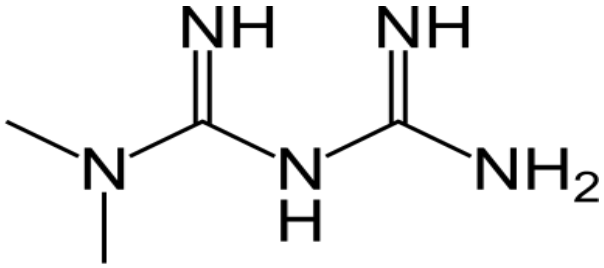

Fig 1: Structure of Metformin.

Vildagliptin: Vildagliptin (previously LAF237, trade names Galvus, Zomelis,) is an oral anti-hyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin inhibits the inactivation of GLP-1[17, 18] and GIP [18] by DPP-4, 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. The EMEA has also approved a new oral treatment released by Novartis, called Eucreas, a combination of vildagliptin and metformin.[19]

Adverse effects observed in clinical trials include nausea, hypoglycemia, tremor, headache and dizziness. Rare cases of hepatoxicity have been reported.[20] There have been case reports of pancreatitis associated with DPP-IV inhibitors. A group at UCLA reported increased pre-cancerous pancreatic changes in rats and in human organ donors who had been treated with DPP-IV inhibitors.[21, 22] In response to these reports, the United States FDA and the European Medicines Agency each undertook independent reviews of all clinical and preclinical data related to the possible association of DPP-IV inhibitors with pancreatic cancer. In a joint letter to the New England Journal of Medicines, the agencies stated that "Both agencies agree that assertions concerning a causal association between incretin-based drugs and pancreatitis

or pancreatic cancer, as expressed recently in the scientific literature and in the media, are inconsistent with the current data. The FDA and the EMA have not reached a final conclusion at this time regarding such a causal relationship. Although the totality of the data that have been reviewed provides reassurance, pancreatitis will continue to be considered a risk associated with these drugs until more data are available; both agencies continue to investigate this safety signal.[23]

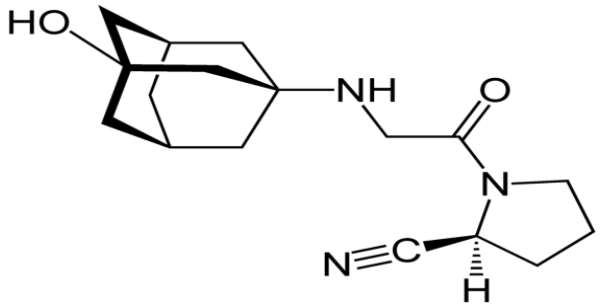

Fig 2: Vildagliptin.

PARAMETERS TO BE CONSIDERED IN THE PROCESS OF VALIDATION

SPECIFICITY: An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure. It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

LINEARITY: A linear relationship should be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

RANGE: The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

ACCURACY: Accuracy should be established across the specified range of the analytical procedure.

PRECISION: Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

REPEATABILITY: Repeatability should be assessed using (a) A minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each) or (b) A minimum of 6 determinations at 100% of the test concentration.

Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

REPRODUCIBILITY

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

MATERIALS AND METHODS

Materials: Metformin and Vildagliptin, Combination Metformin and Vildagliptin tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument: HPLC instrument used was of SHYMADZU HPLC 2965 SYSTEM with manual Injector and UV visible Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin and Vildagliptin solutions.

Methods of Preparation of buffer

Buffer ($0.02KH_2PO_4$): Accurately weighed 1.36gm of potasium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water. (4.0PH)

Standard Preparation: Accurately Weighed and transferred 50mg of Metformin and 5mg of Vildagliptin working Standards into a 10 ml clean dry volumetric flask, add 30ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and

sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Metformin and Vildagliptin are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 125ppm, 250ppm, 375ppm, 500ppm, 625ppm, 700ppm of Metformin and 12.5ppm, 25ppm, 37.5ppm 50ppm, 62.5ppm, 75ppm of Vildagliptin.

Standard Preparation: Accurately Weighed and transferred 50mg of Metformin and 5mg of Vildagliptin working Standards into a 10 ml clean dry volumetric flask, add 30ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Accuracy

Standard Preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 500 mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Sample preparation

50%: 5 tablets were weighed and calculate the average weight of each tablet then 2000mg tablet powder was transferred into a 100mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

100%: 5 tablets were weighed and calculate the average weight of each tablet then 4000mg tablet powder was transferred into a 100mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

150%: 10 tablets were weighed and calculate the average weight of each tablet then 6000mg tablet powder was transferred into a 100mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

METHOD DEVELOPMENT

Many trials were done by changing columns and Mobile phases and were reported belowTrial 1: This trial was run through Altima 150 column with mobile phase composition of 40:20A:40M Buffer, Acetonitrile and Methanol Flow rate set at 1ml/min.

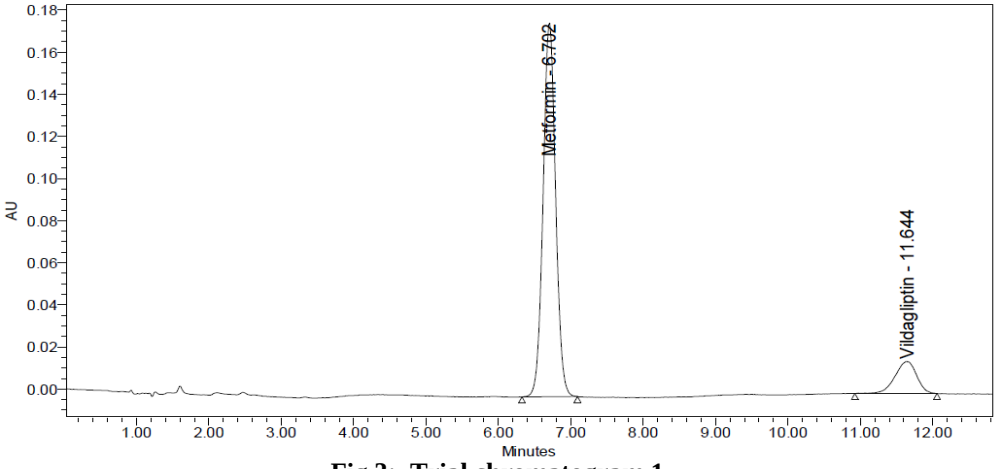

Fig 3: Trial chromatogram 1.

Observation: Metformin and Vildagliptin eluted lately at 6.7min and 11.64min.

Trial 2: This trial was run through Hyber 250mm column with mobile phase composition of 60:40A Buffer and Acetonitrile, Flow rate set at 1ml/min.

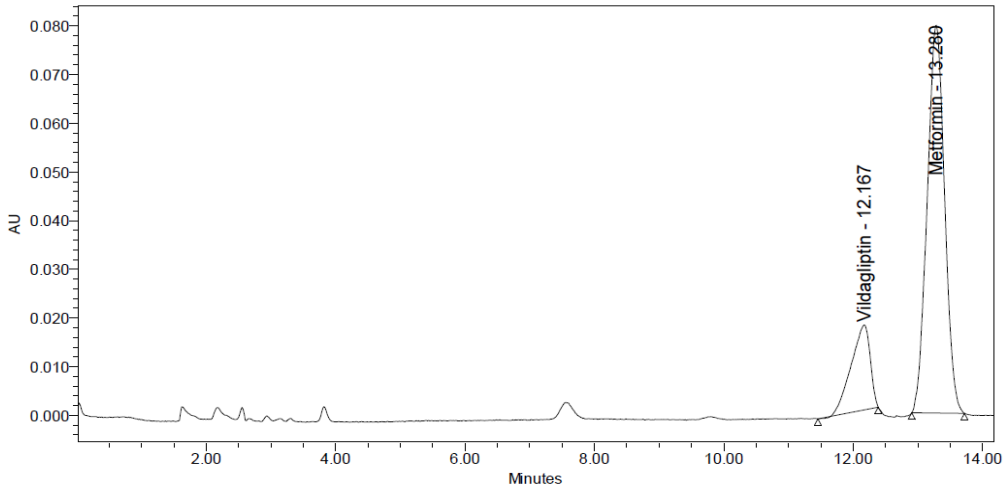

Fig 4: Trial chromatogram 2

Observation: Metformin and Vildagliptin eluted lately with fail in peak shape and Plate count.
Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : ODS (250mm 4.6mm, 5µ)
Buffer used : 0.02KH_2PO_4 pH 4
Mobile phase : Buffer : Acetonitrile (65:35A)
Flow rate : 1ml/min
Diluent : Water and Acetonitrile (50:50)
Wavelength : 210
Temperature : 30°C
Injection Volume : 10µl

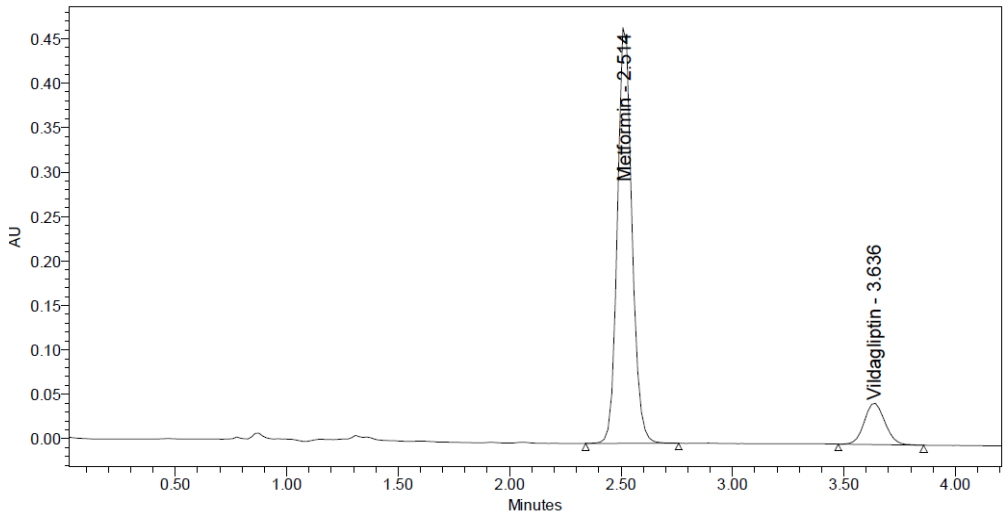

Fig 5: Optimized chromatogram of Metformin and Vildagliptin

RESULTS AND DISCUSSIONS

1. **Systemsuitability:** All the system suitability parameters are within range and satisfactory as per ICH guidelines

Table: 1 System suitability studies of Metformin and Vildagliptin method.

Property	Metformin	Vildagliptin
Retention time (tR)	2.5± 0.3 min	3.6±0.3min
Theoretical plates (N)	7117± 163.48	7358± 163.48
Tailing factor (T)	1.12 ± 0.117	1.11± 0.117

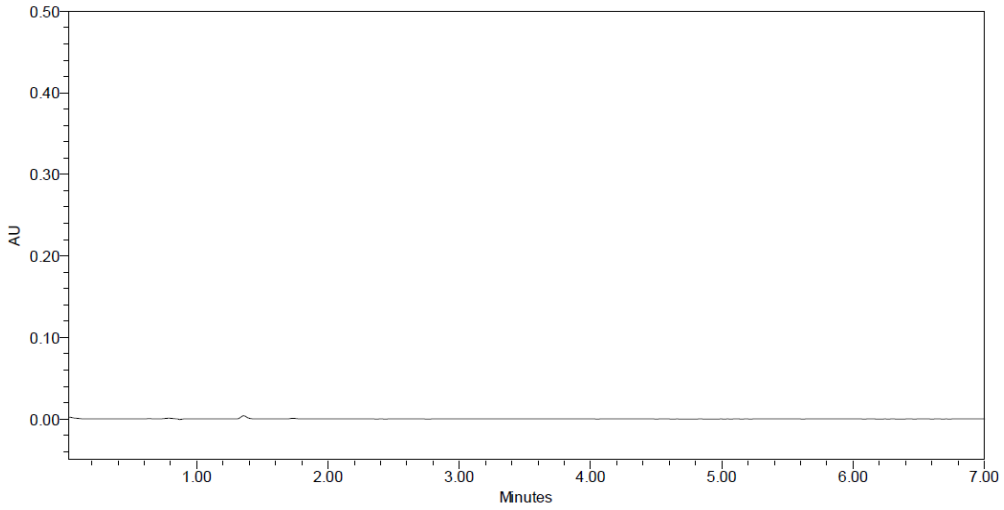

Fig 6: Chromatogram of blank.

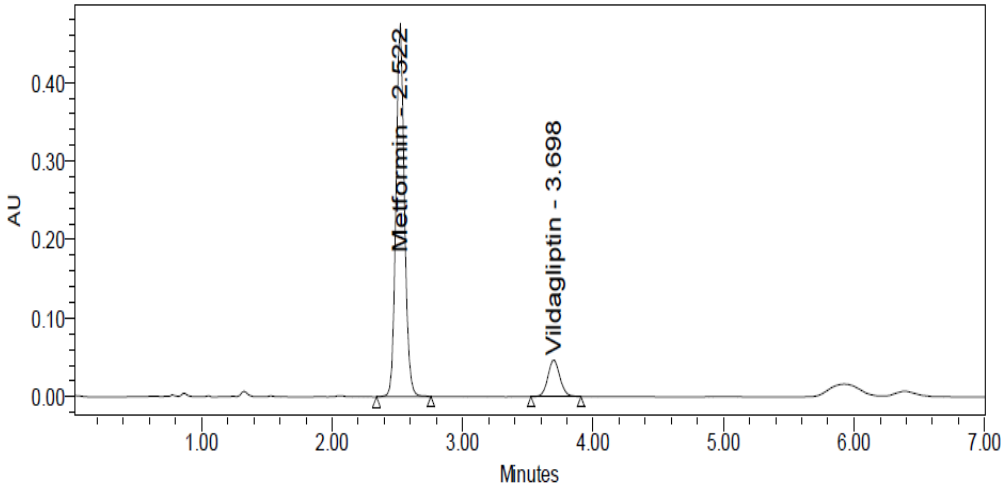

Fig 7: Typical chromatogram of Metformin and Vildagliptin.

2. Linearity: Six Linear concentrations of Metformin(125-750ppm) and Vildagliptin (12.5ppm to 75ppm) are prepared and Injected. Regression equation of the the Metformin and Vildagliptin are found to be, y = 3847.x + 323.7, y = 6074.x + 1247. And regression co-efficient was 0.999.

Table: 2 Calibration data of Metformin and Vildagliptin method.

Sl .no	Concentration Metformin (µg/ml)	Response	Concentration of Vildagliptin (µg/ml)	Response
1	0	0	0	0
2	125	456087	12.5	79411
3	250	972261	25	151553
4	375	1468523	37.5	229876
5	500	1928975	50	305974
6	625	2407857	62.5	378842
7	750	2868997	75	457572

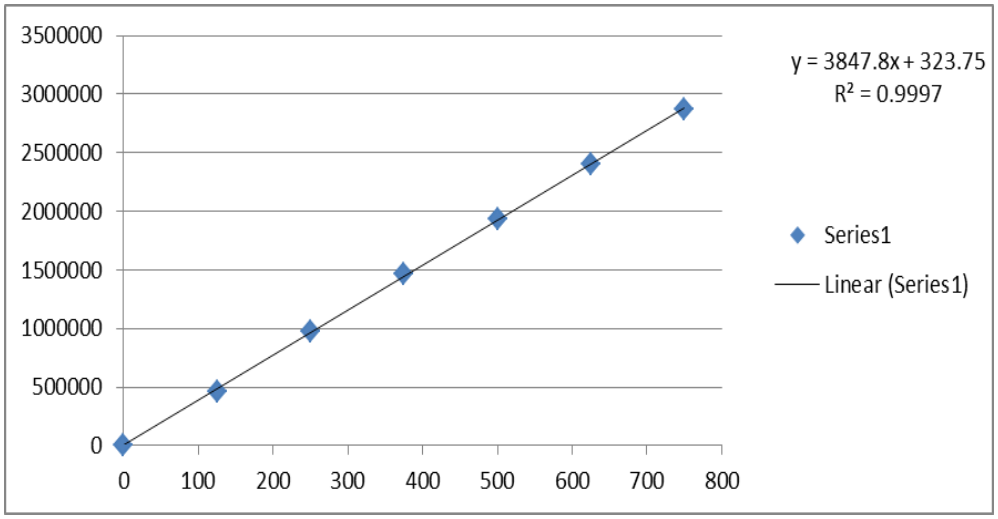

Fig 8: Calibration curve of Metformin.

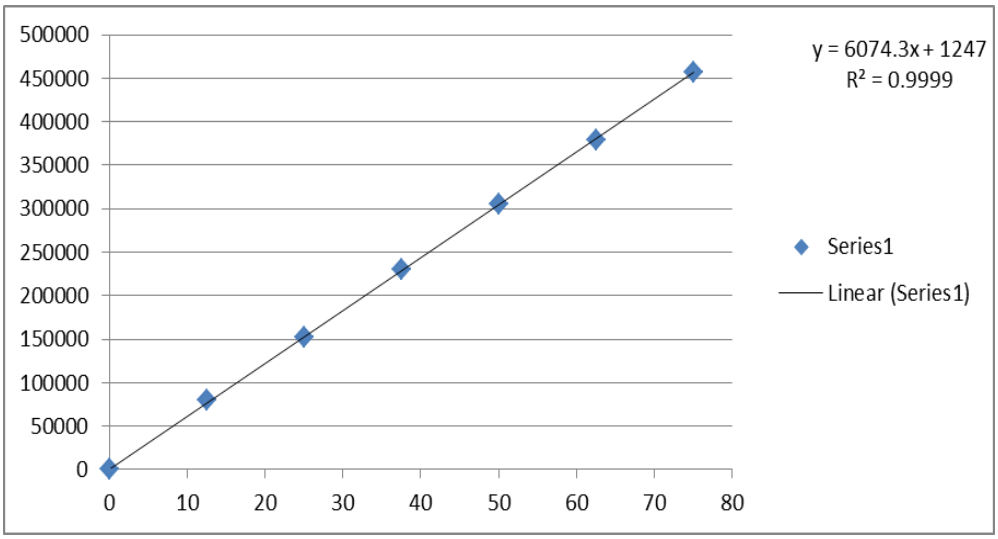

Fig 9: Calibration curve of Vildagliptin.

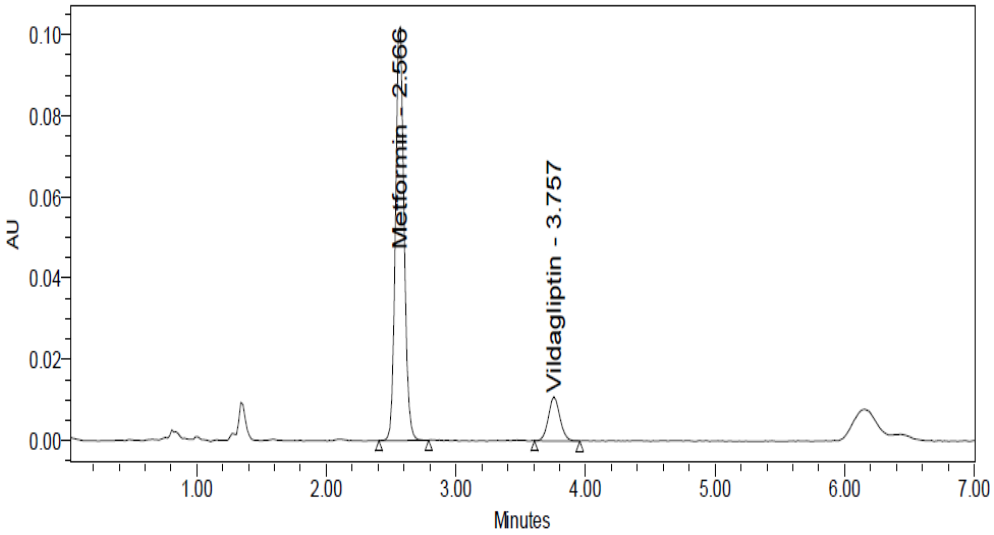

Fig 10: Linearity 25% Chromatogram of Metformin and Vildagliptin method.

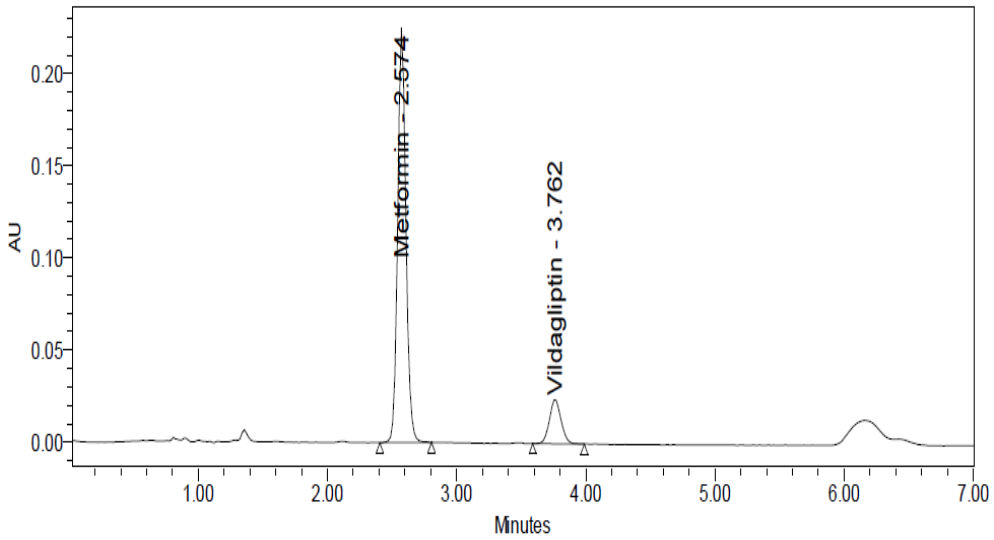

Fig 11: Linearity 50% Chromatogram of Metformin and Vildagliptin method.

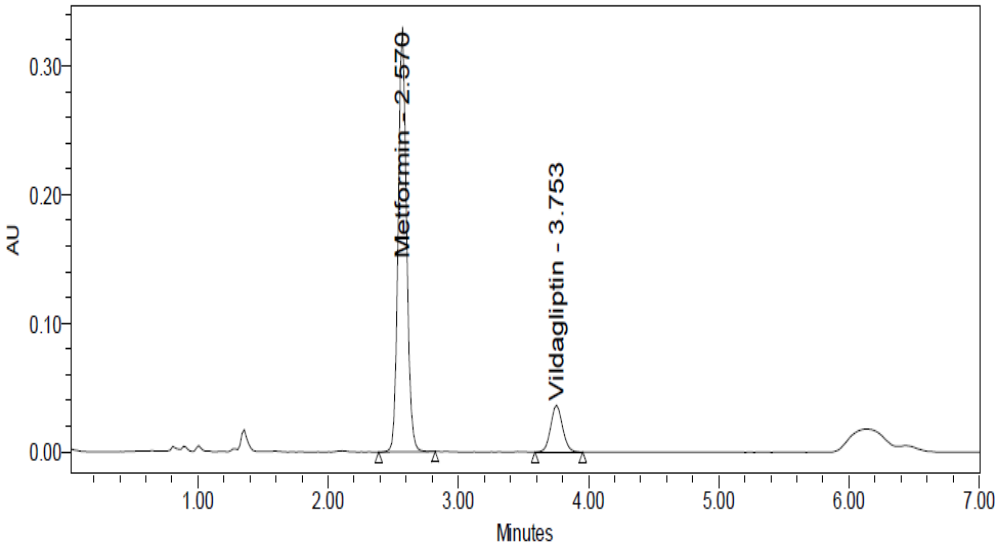

Fig 12: Linearity 75% Chromatogram of Metformin and Vildagliptin method.

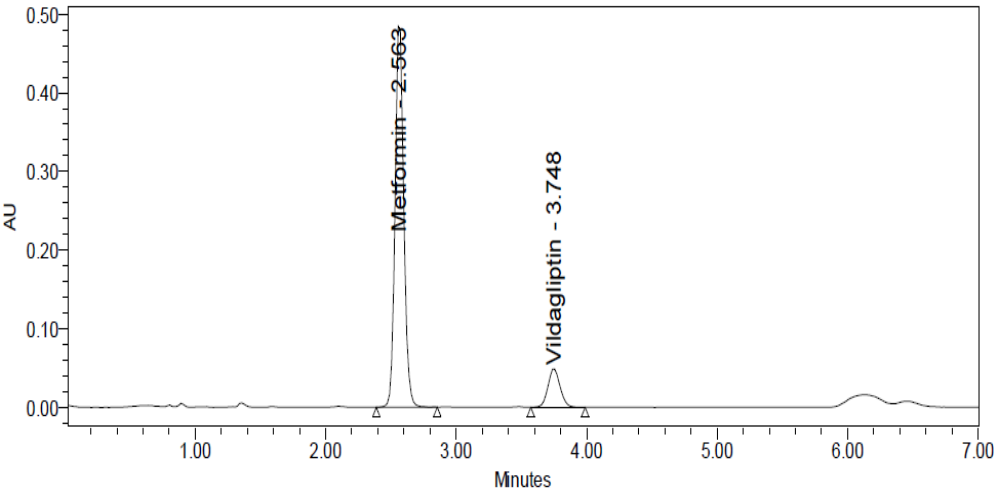

Fig 13: Linearity 100% Chromatogram of Metformin and Vildagliptin method.

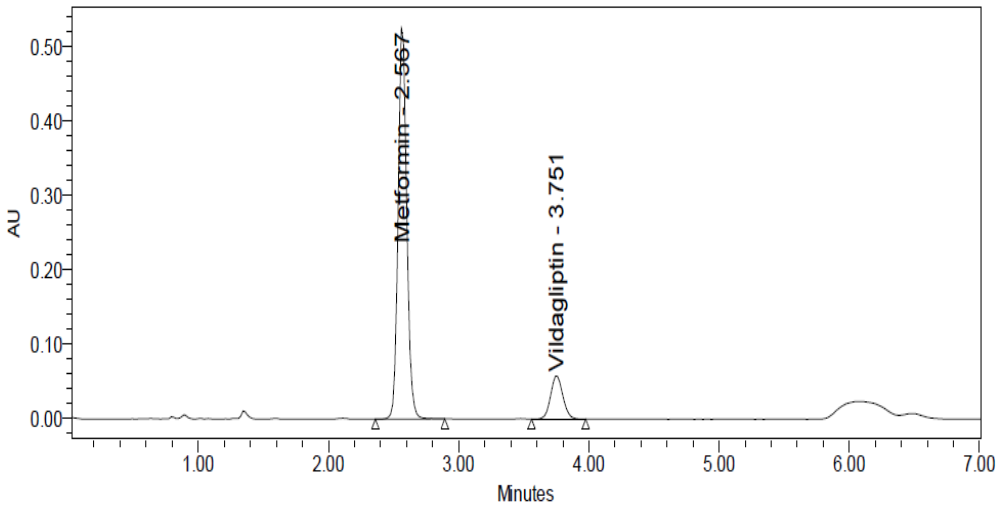

Fig 14 :Linearity 125% Chromatogram of Metformin and Vildagliptin method.

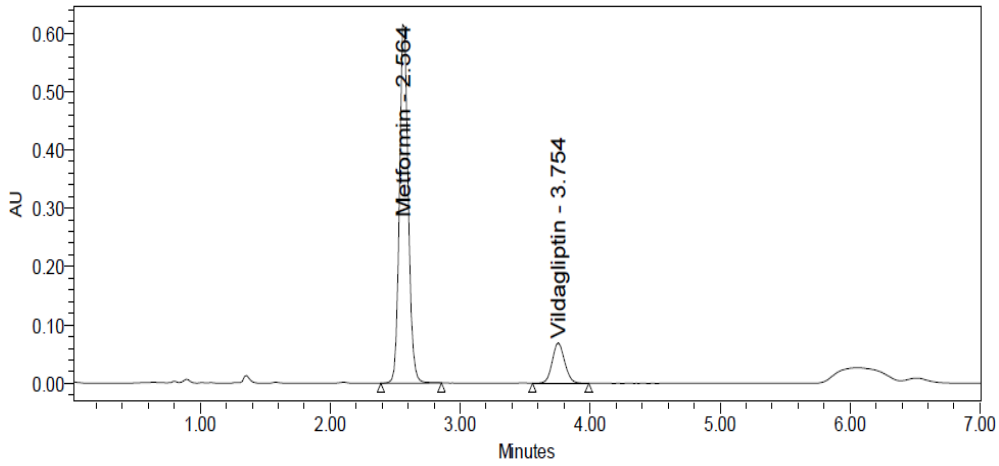

Fig 15: Linearity 150% Chromatogram of Metformin and Vildagliptin method.

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Metformin and Vildagliptin were

Table: 3 Repeatability results for Metformin and Vildagliptin.

Sr. No.	Metformin	Vildagliptin
1	2135455	302457
2	2120878	305863
3	2144225	304692
4	2139285	305812
5	2129205	306639
6	2138190	304689
Mean	2134540	305025
Std. Dev.	8313.7	1465.9
%RSD	0.39	0.5

*Average of six determinations

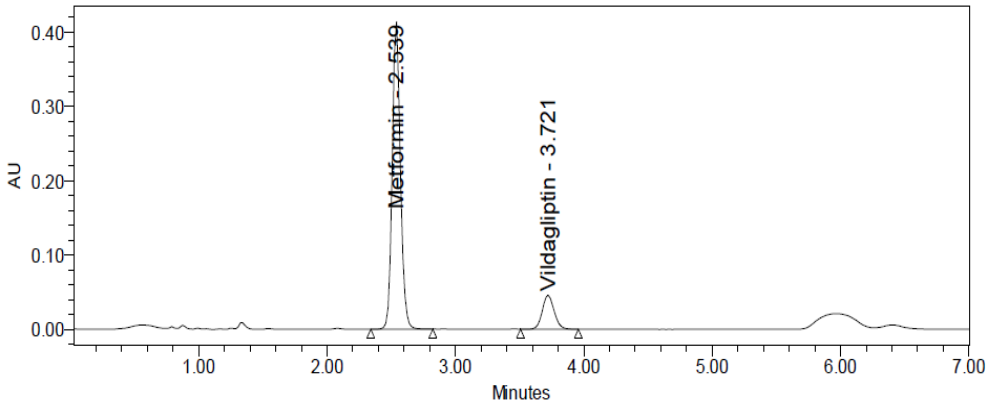

Fig 16: Repeatability Chromatogram of Metformin and Vildagliptin method.

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metformin and Vildagliptin were 0.53 and 0.4.

Table: 4 Inter day precision results for Metformin and Vildagliptin.

Sr. No.	Metformin	Vildagliptin
1	2122807	301089
2	2126181	301105
3	2133854	302890
4	2120237	301887
5	2117517	302583
Mean	2130336	292797
Std. Dev.	2125155	300392
%RSD	6190.43	3793.4

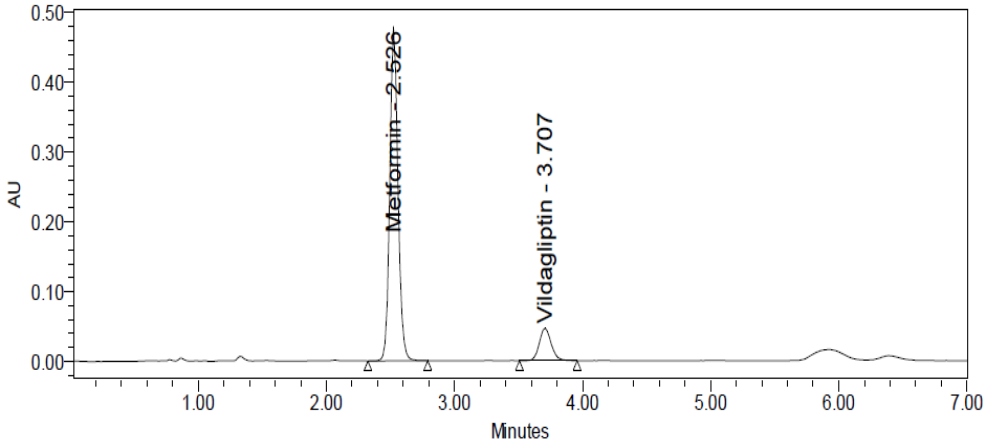

Fig 17: Inter Day precision Chromatogram of Metformin and Vildagliptin method.

3. Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 4

Table: 4. Accuracy results of Metformin and Vildagliptin.

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Metformin	250	249.9	99.99	1.10
	500	506.6	101.32	0.62
	750	750.38	100.05	0.43
Vildagliptin	25	25.26	101.02	0.84
	50	50.23	100.45	0.45
	75	75.13	100.17	0.32

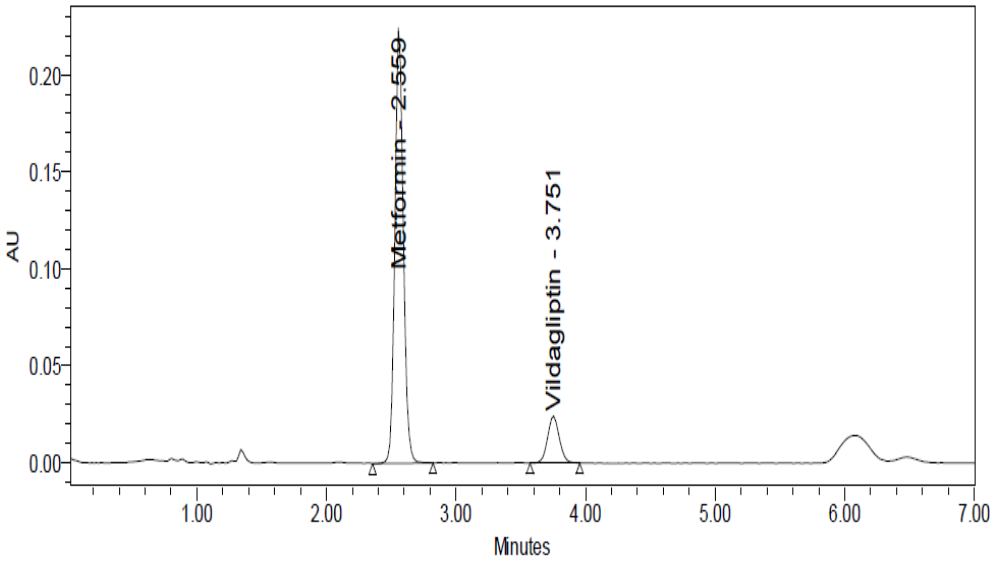

Fig 18: Accuracy 50% Chromatogram of Metformin and Vildagliptin method.

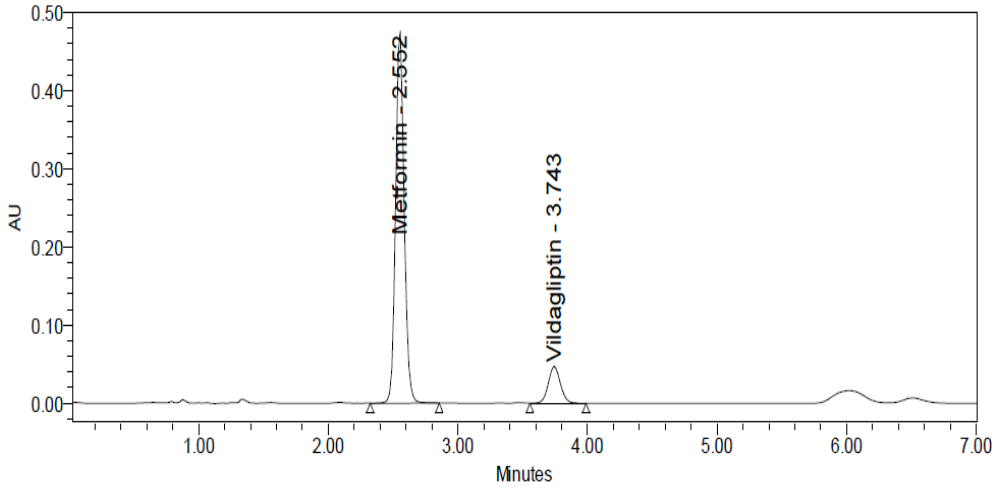

Fig 19: Accuracy 100% Chromatogram of Metformin and Vildagliptin method.

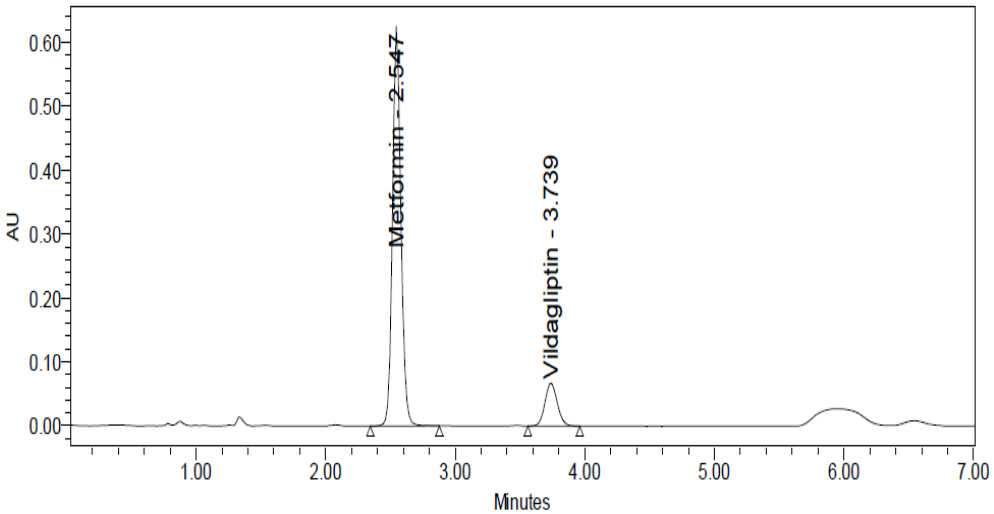

Fig 20: Accuracy 150% Chromatogram of Metformin and Vildagliptin method.

4. LOD: Limit of ditection was calculated by inteMetformin and Vildagliptinpt method and LOD for Metformin and Vildagliptin wre found to be 0.2ppm and 0.6ppm respectively.

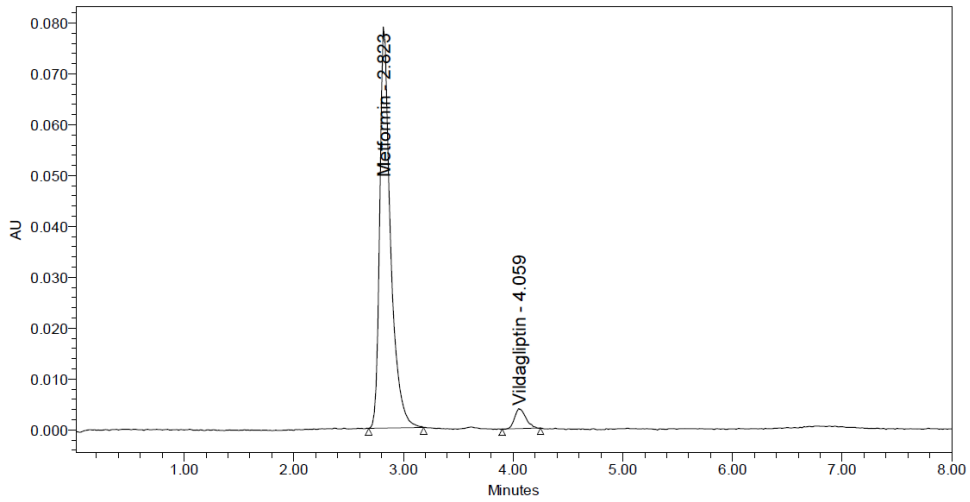

Fig 21 : LOD Chromatogram of Metformin and Vildagliptin method.

5. LOQ: Limit of Quantification was calculated by inteMetformin and Vildagliptinpt method and LOQ for Metformin and Vildagliptin wre found to be 0.8ppm and 2.05ppm respectively.

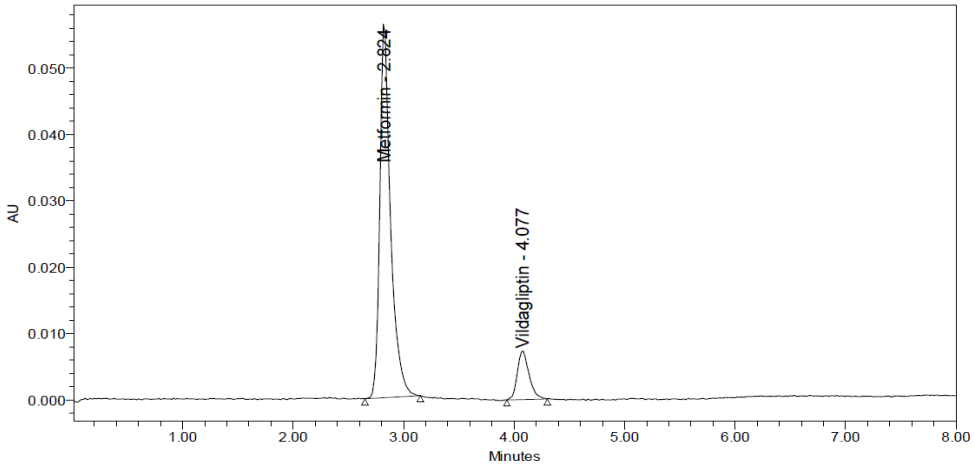

Fig 22 : LOQ Chromatogram of of Metformin and Vildagliptin method.

6. Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Table : 5 Robustness data of Metformin and Vildagliptin method.

S.NO	Robustness condition	Metformin %RSD	Vildagliptin %RSD
1	Flow minus	0.1	0.1
2	Flow Plus	0.1	0.1
3	Mobile phase minus	0.1	0.0
4	Mobile phase Plus	0.3	0.2
5	Temperature minus	0.0	0.3
6	Temperature Plus	0.2	0.1

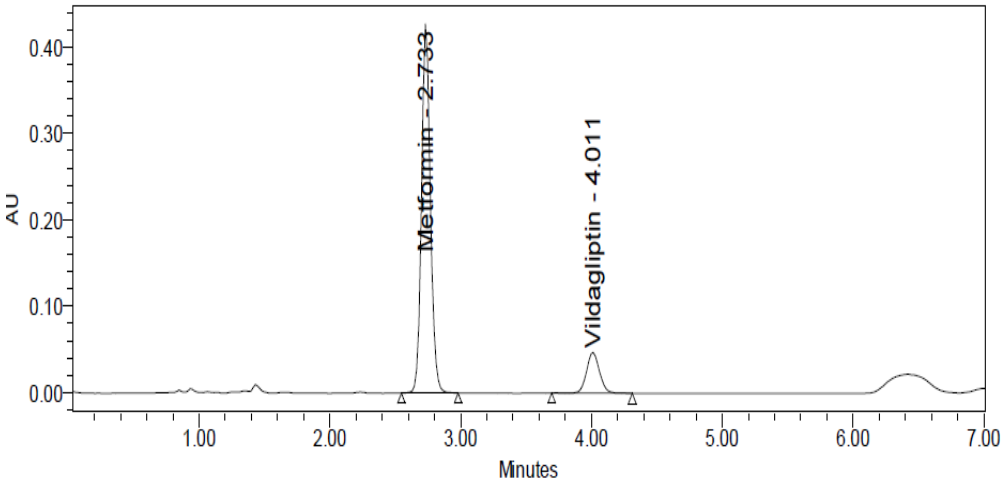

Fig 23: Flow minus Chromatogram of Metformin and Vildagliptin method.

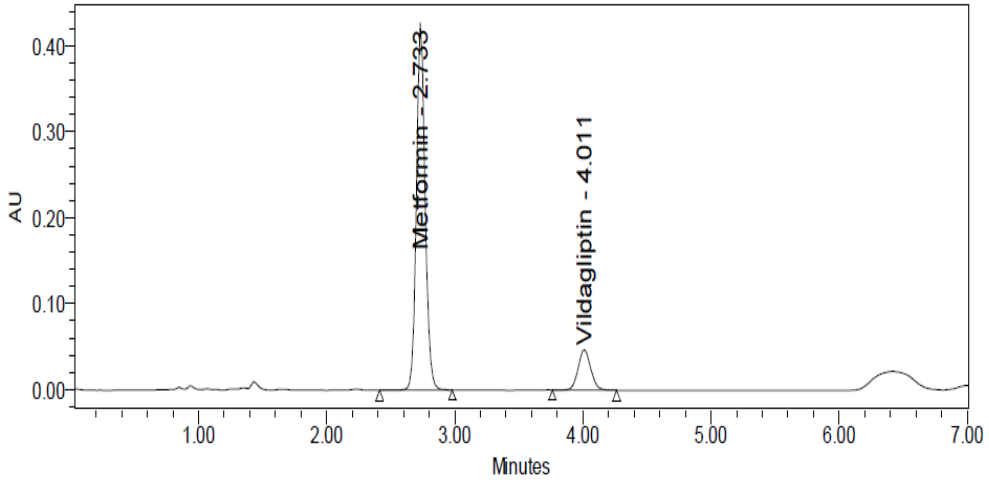

Fig 24: Flow plus Chromatogram of Metformin and Vildagliptin method.

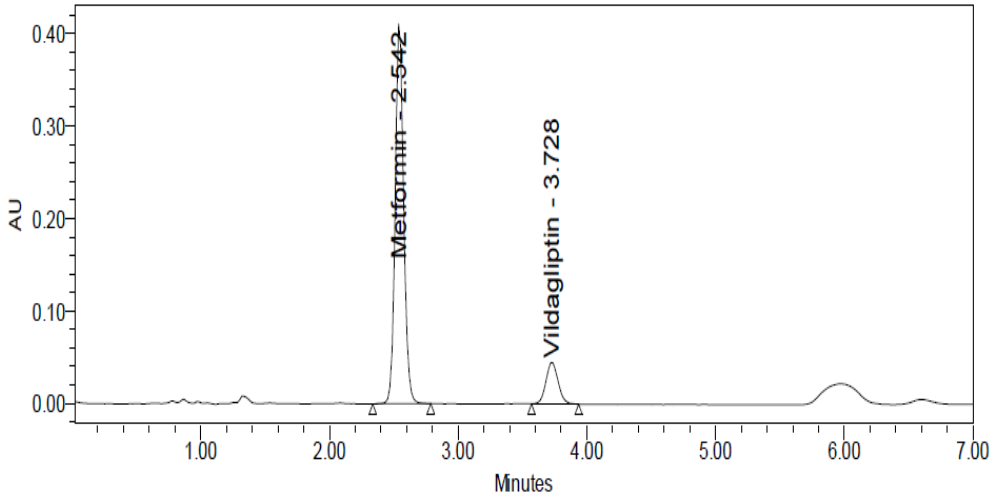

Fig 25: Mobile phase minus Chromatogram of Metformin and Vildagliptin method.

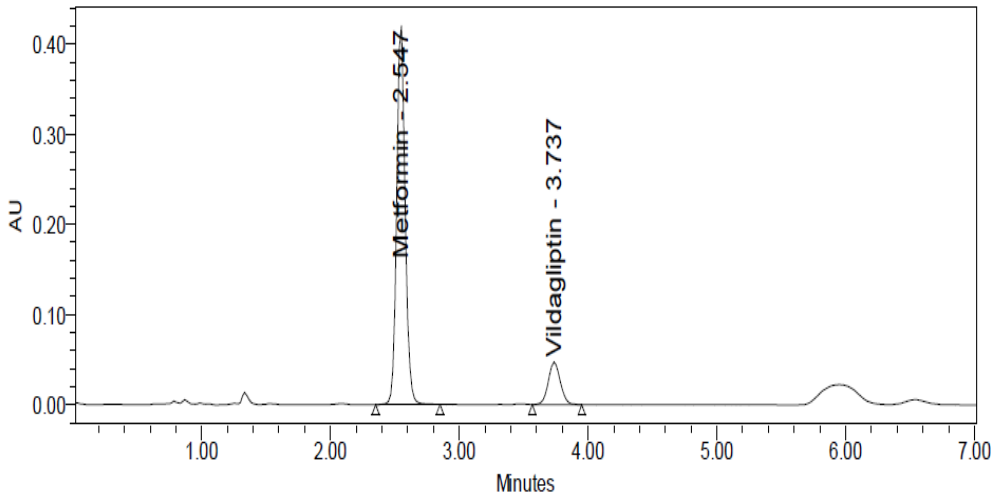

Fig 26: Mobile phase Plus Chromatogram of Metformin and Vildagliptin method.

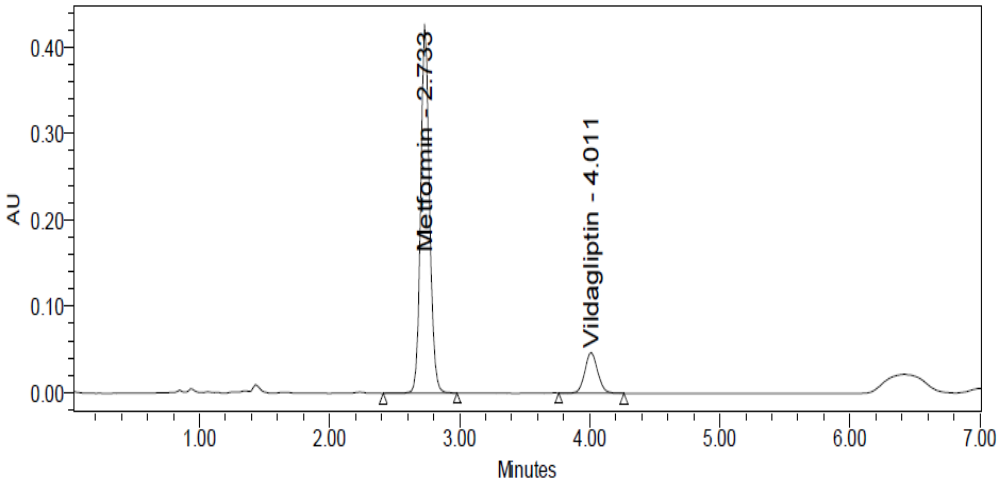

Fig 27: Temperature minus Chromatogram of Metformin and Vildagliptin method.

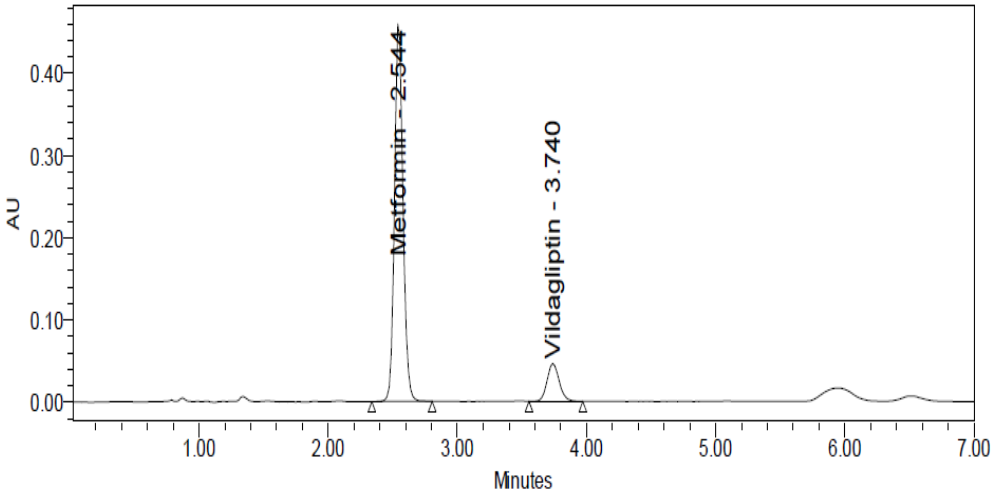

Fig 28: Temperature Plus Chromatogram of Metformin and Vildagliptin method.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.87 and 100.16 for Metformin and Vildagliptin respectively.

Table: 6 Assay of Tablet.

S. No.	Metformin %Assay	Vildagliptin %Assay
1	98.89	100.93
2	100.28	100.94
3	99.94	99.15
4	100.71	100.71
5	100.47	99.29
6	100.36	99.42
AVG	100.11	100.07
STDEV	0.65	0.87
%RSD	0.65	0.87

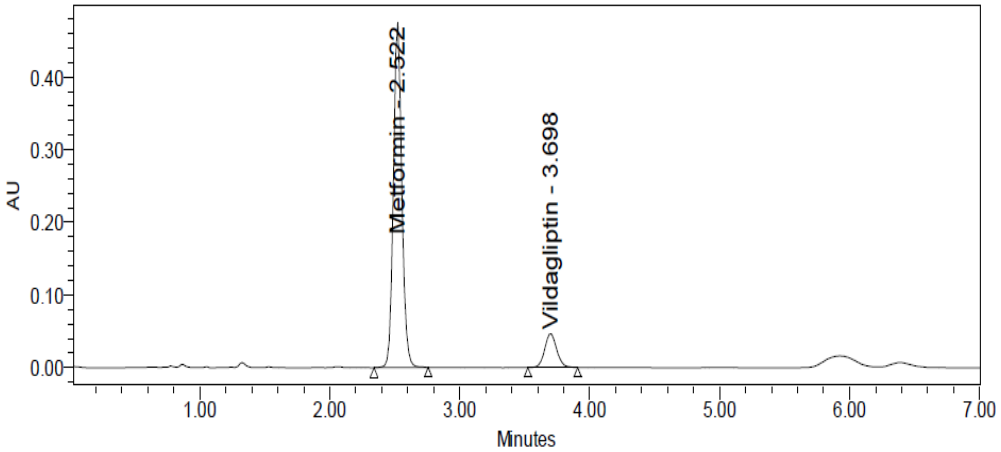

Fig 29: Assay Chromatogram Metformin and Vildagliptin.

SUMMARY

Table: 6.

Parameters	Metformin	Vildagliptin
Calibration range (mcg / ml)	125-750ppm	12.5-75ppm
Optimized wavelength	215nm	215nm
Retention time	2.8min	4min
Regression equation (Y*)	y = 13779x + 1840	y = 16828x + 4143
Correlation coefficient(r2)	0.999	0.999
Precision (% RSD*)	0.65	0.9
% Recovery	99.83%	99.97%
Limit of Detection (mcg / ml)	0.4ppm	0.8ppm
Limit of Quantitation (mcg / ml)	1.3ppm	2.5ppm

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Vildagliptin in Tablet dosage form. Retention time of Metformin and Vildagliptin were found to be 2.8min and 4.0min. %RSD of the Metformin and Vildagliptin were and found to be 0.65 and 0.9 respectively. %Recover was Obtained as 99.83% and 99.97% for Metformin and Vildagliptin respectively. LOD, LOQ values are obtained from regression equations of Metformin and Vildagliptin were 0.4ppm, 1.3ppm and 0.8ppm, 2.5ppm respectively. Regression equation of Metformin is y = 13779x + 1840, and of Vildagliptin is y = 16828x + 4143. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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