ejpmr, 2025, 12(5), 600-611

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A COMPREHENSIVE ION PAIR-FREE CO-ELUTION LIQUID CHROMATOGRAPHY TO DETERMINE THE METFORMIN AND VILDAGLIPTIN, SITAGLIPTIN SIMULTANEOUSLY IN THE PHARMACEUTICAL DOSAGE FORM

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Article Received on 26/03/2025 **Article Revised on 16/04/2025** **Article Accepted on 6/05/2025**

ABSTRACT

An ion pair-free, quick equilibrating, swift and selective HPLC (high performance liquid chromatography) method has been developed to determine the oral antidiabetics: Metformin hydrochloride (MT) and its combination formulation along with Sitagliptin phosphate (ST), Vildagliptin(VG) in single drug formulation and combination solid oral dosage formulations. The isocratic elution mode using solvents A- water and triethylamine adjusted to intermediate pH with ortho-phosphoric acid and B-ethanenitrile as a mobile phase at a flow rate of 1.0 mL/min on Waters Sperisorb ODS2, 250 × 4.6 mm, 5µm HPLC column at 35°C temperature. Area responses were found to be linear, and the correlation coefficients for VG, ST and MT are 0.9996, 0.9995 and 0.9998, respectively. The established limit of detection and limit of quantification for VG was 0.22 µg/ml and 0.90µg/ml, 0.18 µg/ml and 0.64µg/ml for ST, and 0.25 µg/ml and 1.04 µg/ml for MT. The recovery of results is between 98.0 and 102.0 percent with a repeatability of ≤2.0 percent. The method is cost-effective, free of ion-pair reagents and applicable for determining the assay of cited drugs in pharmaceutical preparations and their routine quality control.

KEYWORD:- RP-HPLC; Antidiabetic drugs; Metformin hydrochloride; Sitagliptin phosphate; Vildagliptin.

1. INTRODUCTION

Metformin hydrochloride(MT) is a biguanide-class antihyperglycemic drug with the molecular formula $C_4H_{11}N_5$. HCl and a molecular weight of 165.62 g/mol. It is a white to off-white crystalline powder, freely soluble in water and slightly soluble in alcohol. It is widely used as a first-line pharmacotherapy[1,2] and indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

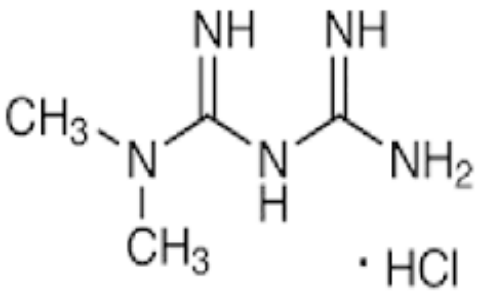


Figure 1: Structure of Metformin HCl.

It is also used in a variety of combination products with other anti-diabetic agents, prescribed as individual tablets and in combination of two DPP-4 inhibitors[3,4,5,6] separately, with Sitagliptin phosphate or Vildagliptin.

Sitagliptin Phosphate (ST) is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor, a small molecule with a molecular weight of 407.314 g/mol.

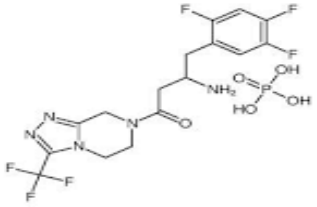


Figure 2: Structure of sitagliptin phosphate.

It is used in conjunction with diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. The effect of this medication leads to glucose-dependent increases in insulin and decreases in glucagon to improve control of blood sugar level.[7,8]

Vildagliptin (VG) is another DPP-4 inhibitor, another small molecule with molecular formula $C_{17}H_{25}N_3O_2$ and a molecular weight of 303.39 g/mol, preferred to use as monotherapy and with metformin.

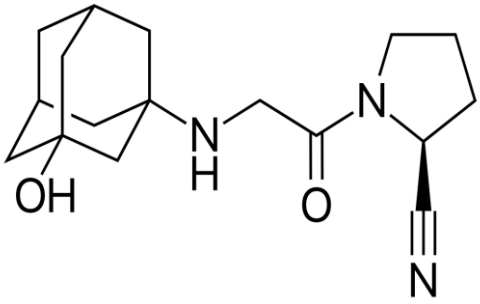


Figure 3: Structure of vildagliptin.

It acts primarily by enhancing pancreatic islet function and promoting glucose-dependent insulin secretion. It prolongs the activity of incretin hormones(GLP-1 and GIP), enhancing glucose-dependent insulin secretion from pancreatin and suppressing glucagon release from alpha cells, causing a lowering of blood glucose without significant hypoglycemia risk.

Diabetes[9,10] is a chronic metabolic disorder characterized by high blood sugar levels, affecting people worldwide. Type 1(TD1) is an autoimmune disease, TD2 is an obesity and lifestyle-linked metabolic disorder, Gestational Diabetes develops in pregnancy, and Latent autoimmune diabetes is an aggregate of TD1 and T2D.

It causes acute complications including cardiovascular disease, nephropathy and related diseases, retinopathy and foot ulcers and amputations.

The treatment intentions are to achieve glycemic control (HbA1c < 7%), manage blood pressure and lipids, and prevent ailments.

The monotherapy limitations are inadequate glycemic control, increased hypoglycemia risk, and weight gain with allied side effects. The combination therapy ameliorates control and lessens the probable complications with minimal complexity. Personalized therapy established on the patient's medical condition eases rigorous control and well-being.

The familiar combination therapies are the Biguanides with DPP-4 inhibitors. It enhances glycemic control, reduces complications, reforms lifestyle, and increases cure alternatives.

A biguanide drug MT is used as individual therapy for regulated control and also utilized in combination with 2 different DPP-4 inhibitors: ST or VG.

This study presents a novel testing application for the co-elution chromatography of MT with ST, VG; widely used antidiabetic agents. Given the varying polarities of these moieties, a method was optimized to ensure selectivity and accuracy.

The desired potency, quality, and efficacy are concerns of formulations due to spurious products in the market. Literature reveals reported RP-HPLC methods[11-22] on conventional C8, C18 columns using different buffers and ion pairing agents.[23-25] MT is hydrophilic, highly polar, and doesn't have adequate retention on these columns even with little organic solvents in aqueous phases. The unretained peaks in the void yield erroneous outcomes.

The methods are outlined with aqueous mobile phases accommodated with ion pairing agents and solvents demerits in chromatography, attributed to leisurely equilibration and troubled gradient elution. The extensive column flushing cannot remove it totally and needs to have dedicated columns.

The Ion pair methods are more complex than RP applications and are non-compatible with mass spectrometry (MS). Several methods yield inconsistent, erratic results, incapable of separating oppositely charged analytes. Demonstrating its effectiveness necessitates multiple screening and studies to establish the ideal conditions.

The isocratic separation of MT and VG is intricate due to their close pKa[26] values, a key physicochemical property that indicates whether a compound can receive or donate a proton (H+). It is calculated as the negative logarithm of a compound's acid dissociation constant (Ka). The pKa values are reported in Table 1.

Table 1: Drugs pKa.

Drug name	pKa Property	Value	Reference
Metformin HCl	Strongest basic	12.33	Drugbank/ Chemaxon
Vildagliptin	Strongest basic	9.03	
Sitagliptin Phosphate	Strongest basic	8.78	

The chromatography is established by reverse phase non-polar Sperisorb ODS2 column and a mobile phase(MP) that balances polar and non-polar elution, facilitating the resolution of these drugs within a reasonable runtime. It has demonstrated distinctive accuracy with validation metrics meeting regulatory expectations.

2. MATERIALS AND METHOD

Reagents and Chemicals

Samples of MT, VG, and ST API's were arranged from various vendors and applied as a working standard. The single drug tablet formulation of Vildagliptin tablets 50 mg, Metformin hydrochloride tablets 500 mg and Sitagliptin tablets 50 mg and the combination tablets of MT with DPP-4 class, viz: Vildagliptin tablets 50 mg and Metformin hydrochloride tablets 500 mg, Sitagliptin tablets 50 mg and Metformin hydrochloride tablets 500 mg; were acquired from nearby medical shops. Triethylamine, o-phosphoric acid, and purified water of Merck were used. The ethanenitrile procured from Advent chem.

2.1 Instruments and equipment

HPLC instrument of the Shimadzu, Model LC2010 was used in the experiment along with the pH meter, analytical balance, and ultrasonic bath during preparation.

Chromatographic conditions on HPLC

The method development was executed on a reverse phase non-polar Waters Sperisorb ODS2 column with isocratic elution mode. The mobile phase was prepared by a mixture of buffer(A) and Ethanenitrile(B) at a ratio of 50:50. As a buffer, 1000mL of water was measured, 1 mL of triethylamine and pH set to 6.8 with 10% H_3PO_4. The flow rate was set at 1.0 mL/min on Sperisorb ODS2, 250 × 4.6 mm, 5µm HPLC column at 35°C. The autosampler temperature was set at 30°C with 20µL injection, and the UV detector was set at 215nm.

The isosbestic point is set to 215 nm concerning the relevant spectrum, the absorbance of each drug, and the content of MT, VG, and ST in the test sample.

UV Spectrums-λmax

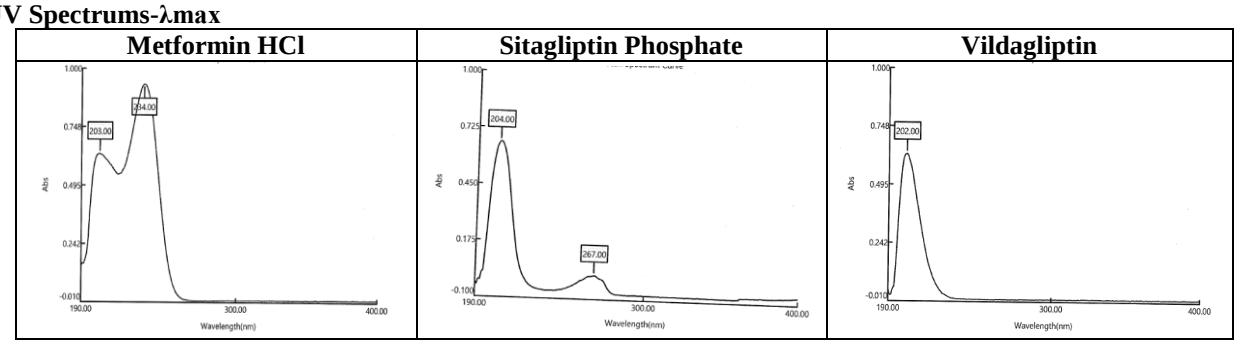


Figure 4: Spectrum of metformin, sitagliptin and vildagliptin.

Method of analysis

An isocratic mode, single method of analysis is developed for three different drug formulations as individual tablets and two different combinations of drugs with MT as common drug. The standards are prepared individually for each drug and also in combination with all three drugs. The sample solutions are prepared for all three drugs as individual tablets and two combinations as they appear in respective combination tablet dosages. End users can select individual or combination drugs according to the requirement and prepare the standard and sample accordingly.

Preparation of diluent

Water and ethanenitrile at a ratio of 50:50 were used as diluent and injected into chromatography as blank.

Preparation of MT standard (500 ppm)

Weighed accurately about 50 mg of metformin standard in 100 mL volumetric flask and dissolved in 70 mL of diluent with intermittent shaking and diluted up to the mark with diluent.

Preparation of VG standard (50 ppm)

Weighed accurately about 50 mg of VG standard in 100 mL volumetric flask and dissolved in 70 mL of diluent with intermittent shaking and diluted up to the mark with diluent. Further, 5 ml of stock solution was diluted to 50 ml with diluent.

Preparation of ST standard (50 ppm)

Weighed accurately about 50 mg of ST standard in 100 mL volumetric flask and dissolved in 70 mL of diluent with intermittent shaking and diluted up to the mark with diluent. Further, 5 ml of stock solution was diluted to 50 ml with diluent.

Estimation of MT from single component tablet dosage form (500 ppm)

10 intact tablets were crushed to fine powder and transferred equivalent to 500 mg of VT in 100 ml volumetric flask. About 70 ml of diluent was added to it and swirled to make it homogeneous. Sonicated for 30 minutes with intermittent shaking, cooled to room temperature, and diluted up to the mark with diluent. Filtered through 0.45 um nylon membrane paper, a further 5 ml of stock solution was diluted to 50 ml with diluent and injected onto HPLC.

Estimation of VG from single component tablet dosage form (50 ppm)

10 intact tablets were crushed to fine powder and transferred equivalent to 50 mg of VG in a 100 ml

volumetric flask. About 70 ml of diluent was added to it and swirled to make it homogeneous. Sonicated for 30 minutes with intermittent shaking, cooled to room temperature and diluted up to the mark with diluent. Filtered through 0.45 um nylon membrane paper, a further 5 ml of the stock solution was diluted to 50 ml with diluent and injected onto HPLC.

Estimation of ST from single component tablet dosage form (50 ppm)

10 intact tablets were crushed to fine powder and transferred equivalent to 50 mg of ST in a 100 ml volumetric flask. About 70 ml of diluent was added to it and swirled to make it homogeneous. Sonicated for 30 minutes with intermittent shaking, cooled to room temperature and diluted up to the mark with diluent. Filtered through 0.45 um nylon membrane paper, a further 5 ml of the stock solution was diluted to 50 ml with diluent and injected onto HPLC.

Estimation of MT and VT in combination tablet dosage form (500 ppm & 50 ppm)

10 intact tablets were crushed to fine powder and transferred equivalent to one tablet in a 100 ml volumetric flask. About 70 ml of diluent was added to it and swirled to make it homogeneous. Sonicated for 30 minutes with intermittent shaking, cooled to room temperature and diluted up to the mark with diluent. Filtered through 0.45 um nylon membrane paper, further 5 ml of the stock solution was diluted to 50 ml with diluent and injected onto HPLC.

Estimation of MT and ST in combination tablet dosage form (500 ppm & 50 ppm)

10 intact tablets were crushed to fine powder and transferred equivalent to one tablet in 100 ml volumetric flask. About 70 ml of diluent was added to it and swirled to make it homogeneous. Sonicated for 30 minutes with intermittent shaking, cooled to room temperature and diluted up to the mark with diluent. Filtered through 0.45 um nylon membrane paper, further 5 ml of the stock solution was diluted to 50 ml with diluent and injected onto HPLC.

3. RESULTS AND DISCUSSIONS

Method Development and Optimisation

MT tablet formulations are available as single drug tablets for monotherapy treatment and along with other antidiabetic drugs. In the present work, two commonly prescribed drugs, VT and ST, along with MT are considered. Apt and swift method that would adapt combination drugs would help the analyst to estimate the contents of drugs in available combinations by single method. Hence, considering the solubility and physicochemical characteristics, this method was developed. A ion-pair free mobile phase consisting of water, triethylamine and orthophosphoric acid mixed with ethanenitrile at the ratio of 50:50 was prepared. Different HPLC columns were studied to achieve the desired resolution with a shorter run time. The method was optimised finally on Sperisorb ODS2 column to achieve an acceptable separation of all drugs that too in a shorter run time. The wavelength selected was such that no interference would be observed from diluent and drugs at the retention of each other. The elution was swift, and the total run time was 15 minutes on HPLC with the well-resolved peaks due to VG, ST, and MT with respective retention times around 4.5, 7.5, and 11.0 minutes.

Method validation

The validation of an analytical method is the process by which it is established, by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical applications.

The developed method for individual drug and for combination tablet formulation was validated for the assay test of MT, VG, and ST in the tablet product. The validation was executed in line with the parameters indicated in ICH guidelines.[27,28] The validation parameters that consisted of only standards were executed in common for all drugs, whereas the remaining parameters that need to be performed on samples were executed separately on the individual tablet product and each combination.

Validation activity was designed to cover the following parameters as indicated in Table 2.

Table 2: Validation activity design.

Type of analytical procedure characteristics	Quantitative
• System suitability	Yes
• Specificity	Yes
• Precision	Yes
• Method precision	Yes
• Intermediate precision	Yes
• Linearity and Range	Yes
• Accuracy (Recovery)	Yes
• Robustness	Yes

Specificity

Initially, the diluent was injected as a blank and the mobile phase was injected to check possible interference. The specific and selective nature of the method for MT, VG, ST was proved by injecting each drug separately in a single injection (Figures 5-10). The samples were then injected for individual and combination tablets. No interference for the diluent, placebo and each API was found at the retention time of the other API (Table 3). Hence, the method is selective for assay of all three drugs at 215 nm.

Table 3: Specificity summary for retention time of analyte.

Sr. No.	Sample Name	Peak Name	Peak at 4.60 min	Peak at 7.50 min	Peak at 10.80 min	Remark
1	Diluent as blank	Blank	No peak	No peak	No peak	No interference
2	Placebo solution	Placebo	No peak	No peak	No peak	No interference
3	Standard VT solution	Vildagliptin	4.60	No peak	No peak	Peak at 4.60 minutes specific to VT
4	Standard ST solution	Sitagliptin	No peak	7.501	No peak	Peak at 7.50 minutes specific to ST
5	Standard MT solution	Metformin	No peak	No peak	10.85 min	Peak at 10.85 minutes Specific to MT
6	Mix standard VT, ST, and MT solution	Vildagliptin, Sitagliptin, Metformin	4.594	7.493	10.90	All 3 peaks are well resolved, specific

Diluent as blank

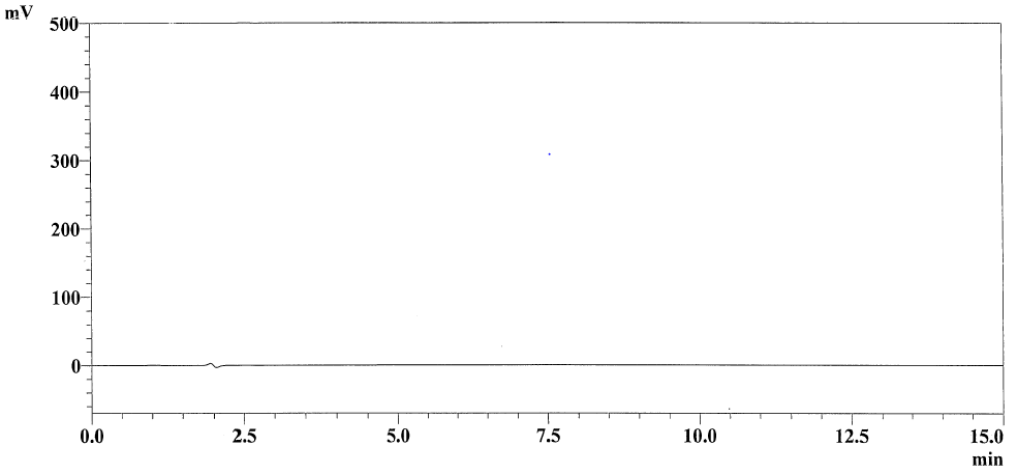


Figure 5: Chromatogram of diluent blank.

Placebo

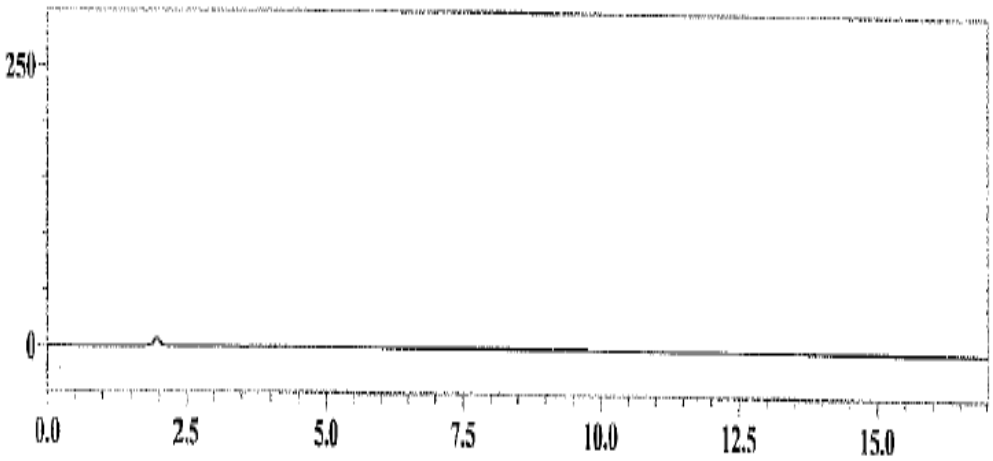


Figure 6: Chromatogram of placebo.

Standard metformin

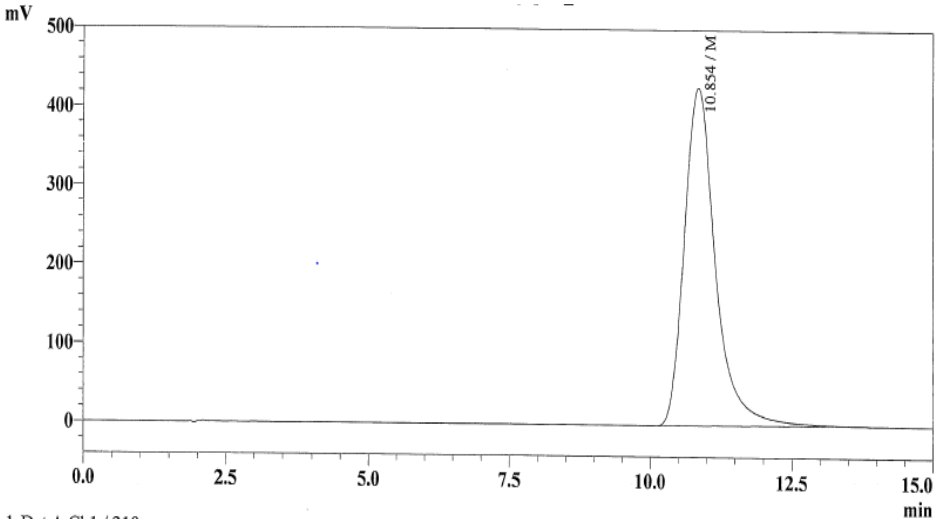


Figure 7: Chromatogram of metformin.

Standard vildagliptin

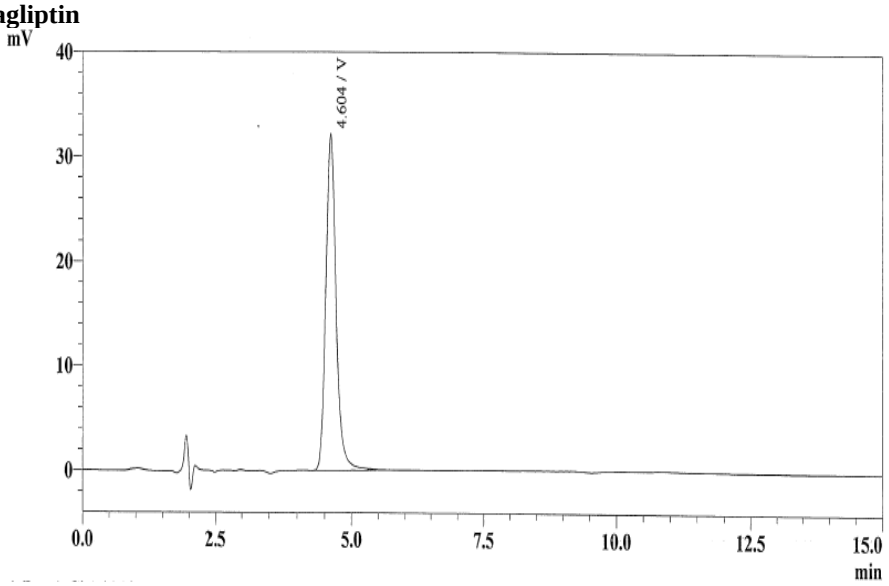


Figure 8: Chromatogram of vildagliptin.

Standard sitagliptin

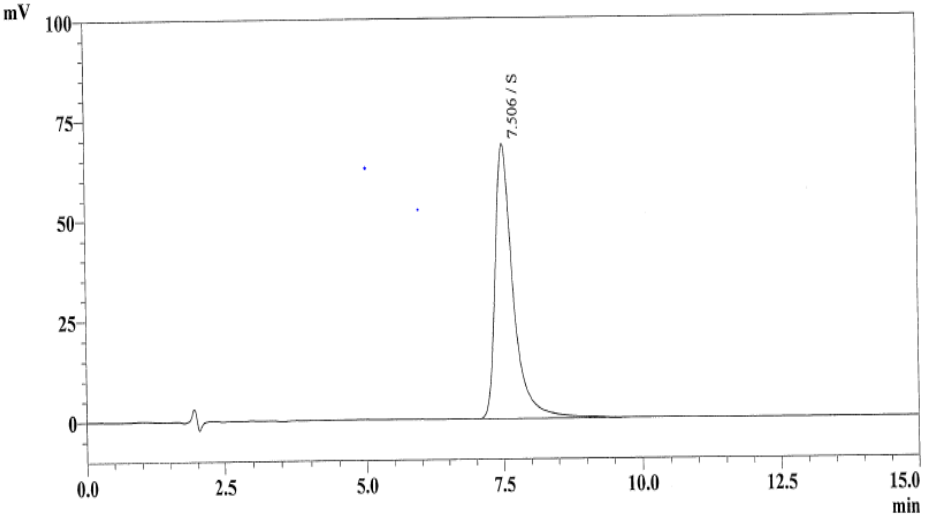


Figure 9: Chromatogram of sitagliptin.

Vildagliptin, Sitagliptin and Metformin chromatogram

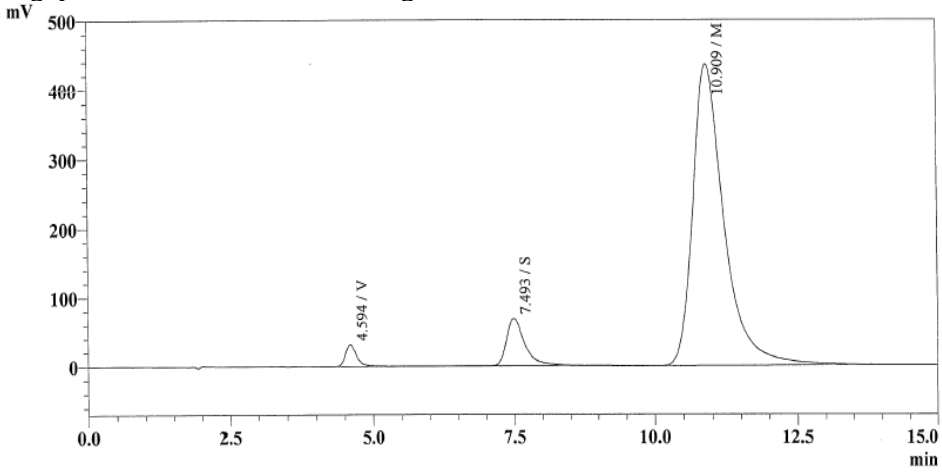


Figure 10: Chromatogram of VT, ST and MT.

Precision

For precision of method, System precision, Method precision and Intermediate precision was carried out. System precision was carried out on five replicate measurements of Metformin and other four API standard solution. For system precision (Table 4), the %RSD for area response should be less than 2%. Method precision was carried out by preparing sample solution six times for each combination tablet of Metformin along with the typical drug (Tables 5,6 and 7). The Assay value and the %RSD were calculated. For Method precision, %RSD should be less than 2%. Intermediate precision (Tables 8,9 and 10) was carried out by carrying out the precision experiment on different days by different analysts. For intermediate precision, the % cumulative RSD of 12 preparations of precision and intermediate precision should not be more than 2%.

Table 4: System precision for three API.

Replicates	VG		ST		MT	
	Retention Time	Area	Retention Time	Area	Retention Time	Area
1	4.59	468708	7.49	1649649	10.91	31593700
2	4.62	472524	7.51	1701386	10.92	31784937
3	4.59	476982	7.49	1698230	10.90	30985931
4	4.58	479852	7.48	1678439	10.92	31765432
5	4.60	469925	7.48	1667469	10.92	31639718
6	4.61	471872	7.51	1695480	10.99	31624392
Average	**4.60**	**473311**	**7.50**	**1681776**	**10.90**	**31565685**
%RSD	**0.30**	**0.90**	**0.20**	**1.20**	**0.30**	**0.90**

Method precision

Table 5: Method precision for Metformin tablets, Vildagliptin tablets, Sitagliptin tablets.

Sample No.	Metformin tablets 500mg		Vildagliptin tablets 50mg		Sitagliptin tablets 50mg	
	Mg/tablet of MT	% Assay	Mg/tablet of VG	% Assay	Mg/tablet of ST	% Assay
Method Precision 1	498.00	99.6	49.45	98.9	50.50	101.0
Method Precision 2	494.00	98.8	49.60	99.2	50.75	101.5
Method Precision 3	496.50	99.3	49.50	99.0	50.50	101.0
Method Precision 4	495.50	99.1	50.35	100.7	50.55	101.1
Method Precision 5	500.50	100.1	49.30	98.6	50.80	101.6
Method Precision 6	497.50	99.5	49.85	99.7	50.60	101.2
Average	**497.0**	**99.4**	**49.7**	**99.4**	**50.62**	**101.2**
%RSD	-	**0.45**	-	**0.76**	-	**0.26**

Table 6: Method precision for Metformin and Vildagliptin combination tablets.

Sample No.	Metformin 500 mg and Vildagliptin 50mg tablets			
	Mg/tablet of MT	% Assay	Mg/tablet of VG	% Assay
Method Precision 1	506.00	101.2	49.85	99.7
Method Precision 2	499.00	99.8	49.45	98.9
Method Precision 3	503.50	100.7	50.25	100.5
Method Precision 4	501.50	100.3	50.55	101.1
Method Precision 5	504.00	100.8	49.75	99.5
Method Precision 6	499.00	99.8	49.65	99.3
Average	**502.17**	**100.4**	**49.92**	**99.8**
%RSD	-	**0.57**	-	**0.82**

Table 7: Method precision for Metformin and Sitagliptin combination tablets.

Sample No.	Metformin 500 mg and Sitagliptin 50 mg tablets			
	Mg/tablet of MT	% Assay	Mg/tablet of ST	% Assay
Method Precision 1	494.50	98.9	50.25	100.5
Method Precision 2	499.00	99.8	49.85	99.7
Method Precision 3	496.50	99.3	50.10	100.2
Method Precision 4	503.00	100.6	49.90	99.8
Method Precision 5	501.00	100.2	50.55	101.1
Method Precision 6	496.50	99.3	49.75	99.5
Average	**498.42**	**99.7**	**50.07**	**100.1**
%RSD	-	**0.64**	-	**0.59**

Intermediate precision

Table 8: Intermediate precision for Metformin tablets, Vildagliptin tablets, Sitagliptin tablets.

Sample No.	Metformin tablets 500mg		Vildagliptin tablets 50mg		Sitagliptin tablets 50mg	
	Mg/tablet of MT	% Assay	Mg/tablet of VG	% Assay	Mg/tablet of ST	% Assay
Intermediate Precision 1	498.00	99.6	49.60	99.2	50.10	100.2
Intermediate Precision 2	501.50	100.3	49.35	98.7	49.90	99.8
Intermediate Precision 3	493.50	98.7	50.05	100.1	50.40	100.8
Intermediate Precision 4	502.50	100.5	50.30	100.6	50.55	101.1
Intermediate Precision 5	506.50	101.3	49.60	99.2	50.20	100.4
Intermediate Precision 6	498.50	99.7	49.40	98.8	49.75	99.5
Average	**500.08**	**100.0**	**49.72**	**99.4**	**50.15**	**100.3**
%RSD	-	**0.89**	-	**0.76**	-	**0.60**

Table 9: Intermediate precision for Metformin and Vildagliptin combination tablets.

Sample No.	Metformin 500 mg and Vildagliptin 50mg tablets			
	Mg/tablet of MT	% Assay	Mg/tablet of VG	% Assay
Intermediate Precision 1	496.00	99.2	50.30	100.6
Intermediate Precision 2	499.00	99.8	49.80	99.6
Intermediate Precision 3	496.00	99.2	49.90	99.8
Intermediate Precision 4	503.00	100.6	50.20	100.4
Intermediate Precision 5	506.00	101.2	50.65	101.3
Intermediate Precision 6	493.50	98.7	49.45	98.9
Average	**498.92**	**99.8**	**50.05**	**100.1**
%RSD	-	**0.95**	-	**0.84**

Table 10: Intermediate precision for Metformin and Sitagliptin combination tablets.

Sample No.	Metformin 500 mg and Sitagliptin 50 mg tablets			
	Mg/tablet of MT	% Assay	Mg/tablet of ST	% Assay
Intermediate Precision 1	505.50	101.1	49.95	99.9
Intermediate Precision 2	492.50	98.5	49.35	98.7
Intermediate Precision 3	494.00	98.8	50.65	101.3
Intermediate Precision 4	504.00	100.8	50.40	100.8
Intermediate Precision 5	498.00	99.6	49.80	99.6
Intermediate Precision 6	501.50	100.3	50.15	100.3
Average	**499.25**	**99.9**	**50.05**	**100.1**
%RSD	**-**	**1.07**	**-**	**0.92**

For system precision, method precision and intermediate precision, all the result values for validation parameters were found to be within acceptance criteria. Hence, it is concluded that the method was precise and rugged.

Linearity and range

For the determination of Linearity and Range of the method, six solutions for VT, MT and seven solutions for ST of different concentrations from 10% to 150% (Figures 11, 12) of the working level of sample for each drug were prepared. The area response of these solutions was recorded after injecting onto the HPLC system. Correlation coefficient, Slope and Intercept were determined by statistical calculations. For a method to be linear within the workable range, the Correlation coefficient should be more than 0.99.

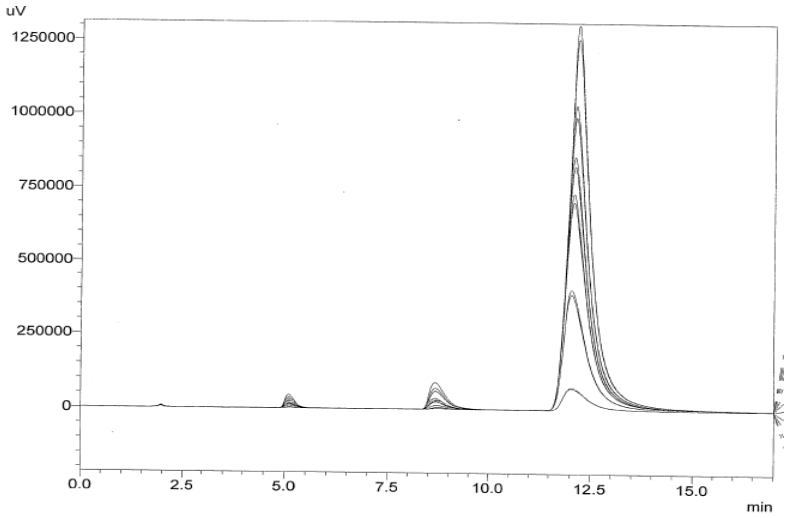
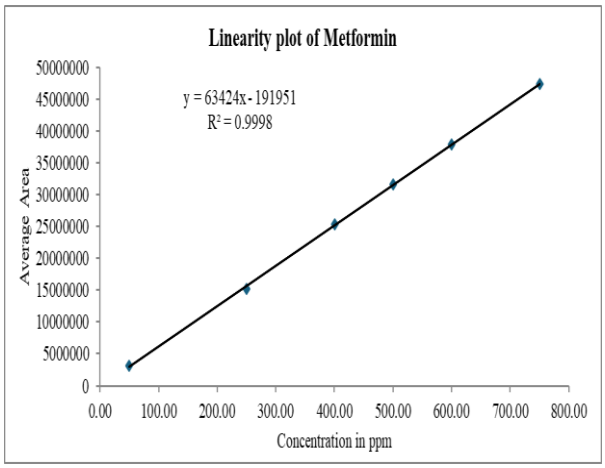
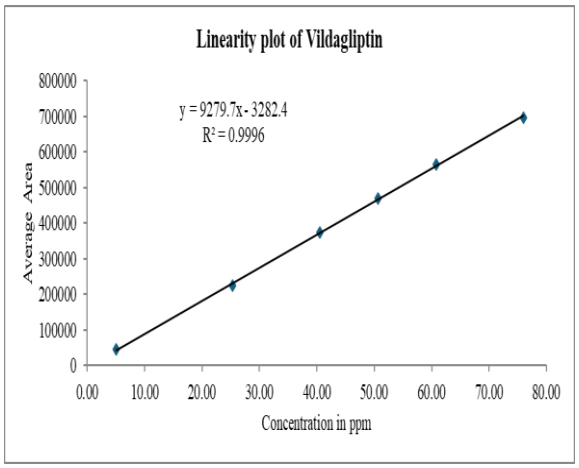
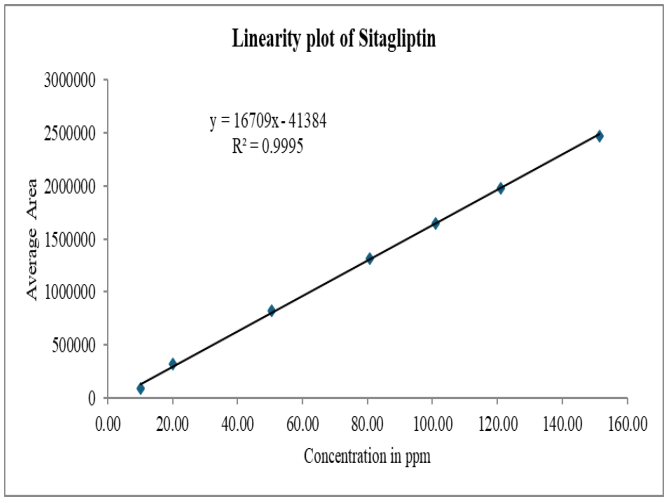


Figure 11: Chromatogram of diluent blank.







Linearity plots of Metformin, Vildagliptin and Sitagliptin
Figure 12: Chromatogram of diluent blank.

From the linearity data and the subsequent statistical analysis, the following results were found, as mentioned below (Table 11).

Table 11: Results obtained from the statistical analysis.

Active component	Working range	Correlation coefficient(R^2)	Slope	Y Intercept
Metformin	10% (50.03 ppm) to 150% (750.41 ppm)	0.9998	63424.1	191951
Vildagliptin	10% (5.07 ppm) To 150% (76.01 ppm)	0.9996	9279.7	3282
Sitagliptin	10% (10.09 ppm) to 150% (151.38 ppm)	0.9995	16708.7	41384

Hence it is concluded that the method is linear within the predefined working range of drugs.

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

From the linearity data prediction and the subsequent experiments, the following results were found, as mentioned below (Table 12)

Table 12

Active component	LOD	LOQ
Metformin	0.25 ppm	1.04 ppm
Vildagliptin	0.22 ppm	0.90 ppm
Sitagliptin	0.18 ppm	0.64 ppm

Accuracy

Accuracy was determined by recovery study of MT and each drug combination (Table 13), at three levels in the range of 80% to 120%. For a method to be accurate, the mean recovery should be between 98.0 to 102.0%.

Table 13: Recovery results for metformin and combination tablet method.

Name of the drug	Recovery levels			Mean recovery
	80%	100%	120%	
Metformin tablets 500 mg				
Metformin	99.1	101.2	98.9	99.7
Vildagliptin tablets 50 mg				
Vildagliptin	100.5	99.9	99.6	100.0
Sitagliptin tablets 50 mg				
Sitagliptin	100.1	100.6	99.5	100.1
Metformin 500 mg and Sitagliptin 50 mg tablets				

Metformin	100.1	99.8	99.3	99.7
Vildagliptin	100.2	101.1	99.7	100.3
Metformin 500 mg and Sitagliptin 50 mg tablets				
Metformin	99.8	99.6	98.9	99.4
Sitagliptin	99.7	100.2	100.7	100.2

Based on the above results, it is proved that the method is accurate for the recovery of MT and the other two drugs, viz: VT and ST, in its single-drug formulation and each combination formulation.

Robustness

The robust method enhances confidence and minimizes the risk of variable outcomes within inter-laboratory studies and conditions with desired outcomes. The robustness needs to be proved by small deliberate change in the experimental condition. The study was performed on both combination products. For robustness, the deliberate changes in HPLC method parameters were done as follows: Change in temperature, Change in wavelength, Change in Flow rate. The results from deliberately changed conditions were compared with as such conditions. For a method to be robust, the absolute difference between as such conditions and deliberately changed conditions should not be more than 2.0%. The results for % difference in Assay values are compiled in below table 14.

Table 14:

Metformin 500 mg and Vildagliptin 50 mg tablets				
Content	**% Assay MT**	**% difference**	**% Assay VG**	**% difference**
As such sample	101.2	-	99.8	-
Change in temperature Low- 30°C	100.8	0.4	99.2	0.6
Change in temperature High- 40°C	101.3	-0.1	100.2	-0.4
Change in wavelength Low- 213 nm	100.6	0.6	98.9	0.9
Change in wavelength High- 217 nm	100.9	0.3	100.4	-0.6
Change in Flow rate Low- 0.8 ml/min	101.6	-0.4	99.7	0.1
Change in Flow rate High- 1.2 ml/min	101.0	0.2	98.9	0.9
Metformin 500 mg and Sitagliptin 50 mg tablets				
Content	% Assay MT	% difference	% Assay ST	% difference
As such sample	100.1	-	100.4	-
Change in temperature Low- 30°C	99.7	0.4	99.6	0.8
Change in temperature High- 40°C	98.9	1.2	101.1	-0.7
Change in wavelength Low- 213 nm	100.4	-0.3	100.9	-0.5
Change in wavelength High- 217 nm	101.3	-1.2	101.2	-0.8
Change in Flow rate Low- 0.8 ml/min	99.8	0.3	99.8	0.6
Change in Flow rate High- 1.2 ml/min	100.3	-0.2	99.6	0.8

As results were within the acceptable criteria even after deliberate change to the experimental conditions, it is proved that the method remained unaffected by small variations of parameter.

CONCLUSION

A single analytical method by HPLC for the determination of Assay of Metformin and its two combination drugs viz: Vildagliptin and Sitagliptin, from single drug tablet and combination tablet was developed and validated. The developed method is without ion-pair reagent, quick equilibrating with consistency in the results, and was found to be specific, accurate, precise, linear, and robust for its intended use in pharmaceutical quality control.

Conflicts of interest

The authors announce no competing financial interest.

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