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An improved validated RP-HPLC method for simultaneous estimation of Metformin Hydrochloride, Sitagliptin phosphate and Dapagliflozin from finish dosage form

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Abstract

A simple and precise reversed-phase high-performance liquid chromatography method was developed and validated for the simultaneous determination of **Metformin (MET) hydrochloride, Sitagliptin phosphate (SITA) and Dapagliflozin (DAPA)** in bulk and pharmaceutical dosage form.

Chromatography was carried out on **Zorobax Eclipse plus Phenyl Hexyl C18 (250 mm × 4.6 mm, 5 µ particle size)** column containing mobile phase of **Buffer: Methanol: Acetonitrile** in the ratio of **40:35:25% v/v.** (1ml of **Triethylamine** is added and **pH** is **adjusted to 3.7** with **0.1% Orthophosphoric acid**) at a flow rate of **1.0 ml/minute**. The analyte was monitored using **Photo Diode Array Detector** (PDA) at **267 nm**.

The retention time was found to be **2.71 minutes, 3.65 minutes and 8.99** minutes for **MET hydrochloride, Sitagliptin phosphate** and **Dapagliflozin** respectively. Validation parameters specificity, linearity, accuracy, precision and robustness have been observed to be desirable over the concentration ranges of **500 µg/ml, 100 µg/ml & 10 µg/ml** for **Metformin (MET) hydrochloride, Sitagliptin phosphate (SITA) and Dapagliflozin (DAPA)** respectively. The method developed has been statistically validated according to ICH guidelines.

The results of analysis have been validated as per **International Conference on Harmonization** (ICH) guidelines. Hence the optimized method can be successfully applied for the simultaneous determination of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin in the routine quality control analysis. Significance of developed method is that, it can be utilized for routine or unknown sample analysis of assay of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin in pharmaceutical dosage form developed by various pharmaceutical industries. The Proposed method was found to be rapid, accurate, precise, specific, robust, rugged and economical.

Keywords: Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin, RP-HPLC

INTRODUCTION:

Metformin (MET) hydrochloride (Fig. 1) is chemically known as (3-(diaminomethylidene)-1,1-dimethylbiguanide; hydrochloride. It has molecular formula $C_4H_{11}N_5$ and molecular weight is 129.16 g/mol. MET is an agent belonging to the biguanide class of antidiabetics with antihyperglycemic activity. MET is the first line agent for the treatment of Type 2 diabetes.

Sitagliptin phosphate (Fig. 2) is an antidiabetic agent which is an oral dipeptidyl peptidase-4 inhibitor which exerts its actions in type 2 diabetes patients by slowing the inactivation of incretin hormones. Sitagliptin (C16H15F6N5 O; Mol. Wt. 407.314 g/mol) is chemically (R)-4-oxo-4- [3-(trifluoromethyl)- 5,6-dihydro[1,2,4] triazolo [4,3- a] pyrazin-7(8H)-yl]-1-(2,4,5-Trifluoro phenyl) butan-2 amine. It stimulates insulin secretion when hyperglycemia is present and inhibits glucagon secretion . Sitagliptin is used along with diet and exercise and sometimes with other medications to lower blood sugar levels in adults with type 2 diabetes (condition in which blood sugar is too high because the body does not produce or use insulin normally). Most experts consider metformin to be the safest medicine for type 2 diabetes because it has been used for many decades, is effective, affordable, and safe. Metformin is recommended as a first-line treatment for type 2 diabetes by the American Diabetes Association (ADA).

Dapagliflozin (DAPA) (Fig. 3) is a selective sodium glucose co-transporter subtype-2 (SGLT-2) inhibitor with antihyperglycemic activity. Its chemical name is (2S, 3R, 4R, 5S, 6R)-2[4-chloro-3-(4-ethoxyphenyl) methyl] phenyl-6-(hydroxymethyl) oxane-3, 4, 5-triol. It has molecular formula of $C_{21}H_{25}ClO_6$ and molecular weight is 408.9 g/mol. DAPA is a SGLT-2 inhibitor indicated for managing diabetes mellitus Type 2.

The combination of Metformin, Sitagliptin, and Dapagliflozin is used to provide a comprehensive approach to managing type 2 diabetes. Each medication addresses different mechanisms of the disease: Metformin improves insulin sensitivity and decreases glucose production. Sitagliptin enhances the incretion system to boost insulin secretion and suppress glucagon release. Dapagliflozin promotes renal glucose excretion. This multi-faceted approach allows for more effective glycemic control, potentially reducing the risk of complications associated with diabetes. The combination therapy can improve patient adherence by reducing the pill burden and simplifying the treatment regimen.

The combination dosage form selected for the present study contains Sita, Dapa and Met in solid oral dosage forms. Recently this combination has been approved by USFDA. The main aim of this study was to develop a stability indicating method for the simultaneous estimation of Sita, Dapa and Met in bulk and to apply the developed method for the quantitative

determination of these drugs from its tablets and the reverse phase high performance liquid chromatography (RP-HPLC) method is chosen. This method is validated as per International Conference on Harmonization (ICH) guidelines. Literature survey revealed that some analytical methods were reported for the estimation of Met, Sita and Dapa individually or in combination with other drugs, by HPLC analytical method. Number of stability indicating RP-HPLC method was reported for estimation of these drugs. Now a day, stability indicating method as important regulatory and cGMP point of view to assess the drug stability. In the present study, it was tired to develop stability indicating RP-HPLC method to determine possible degradation products of Met, Sita and Dapa.

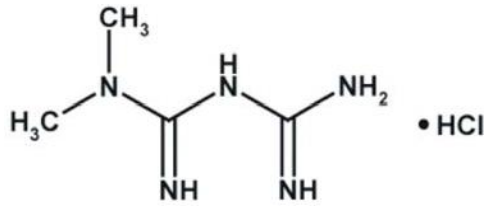


Fig. 1: Structure of Metformin

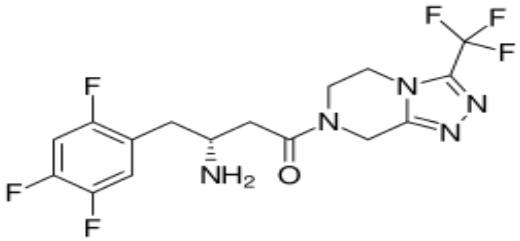


Fig. 2: Structure of Sitagliptin phosphate

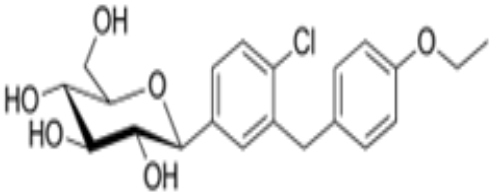


Fig. 3: Structure of Dapagliflozin

MATERIAL AND METHOD:

Instruments:

Chromatographic separation is achieved using HPLC System (Agilent HPLC 1100Series) containing Quaternary pump solvent manager, autosampler and PDA detector. The output Signal is monitored and processed using Ezchrom Elite Software. A Zorobax eclipse Plus Phenyl Hexyl column (250 x 4.6mm, Id 5µ) is used as stationary phase. Isocratic mode is used for the elution.

Chemicals and Reagents:

Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin as active pharmaceutical

ingredients (API).

Methanol, Acetonitrile, Triethylamine & 0.1% Orthophosphoric acid, HPLC grade Water and potassium dihydrogen ortho-phosphate buffer of HPLC grade.

Preparation of Buffer pH 3.7:

Dissolve 3.4 g of potassium dihydrogen ortho-phosphate in 1000 mL of HPLC grade water. 1mL **Triethylamine** is added. The solution is **s**onicated to dissolve and pH is adjusted to 3.7 with 0.1% ortho phosphoric acid and filtered through 0.45µ filter.

Preparation of Mobile phase (Diluent):

Initially, trials were carried out using various solvents in various proportions on HPLC to obtain the desired peak shape for drug. After few trials **Buffer: Methanol: Acetonitrile (40:35:25% v/v)** was fixed as the mobile phase which gave acceptable peak parameters.

400mL of Buffer pH 3.7, 350mL Methanol & 250 mL Acetonitrile Mixed well and degassed.

Chromatographic Conditions:

HPLC System	**:**	Agilent 1100 Series with PDA Detector
Mobile Phase	**:**	Buffer **:** Methanol **:** Acetonitrile (40:35:25% v/v)
Column Used	**:**	Zorobax eclipse Plus Phenyl Hexyl (250 x4.6mm, id 5µ)
Column Temperature	**:**	30°C
Flow Rate	**:**	1.0mL/Minutes
Injection Volume	**:**	20µL
Detection Wavelength	**:**	267nm
Mode	**:**	Isocratic elution
Run Time	**:**	15 minutes

Preparation of Stock (Standard) Solution:

Weigh and transfer about **100 mg** of **standard Metformin Hydrochloride** in 100 mL volumetric flask. Add about 30 mL of diluent and sonicate to dissolve. Cool the solution and

dilute up to the mark with diluent (1mg/mL=1000ppm).

Weigh and transfer about **100 mg** of **standard Sitagliptin phosphate** in 100 mL volumetric flask. Add about 30 mL of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm).

Weigh and transfer about **100 mg** of standard **Dapagliflozin** in 100 mL volumetric flask. Add about 30 mL of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm).Pipette out standard 10mL of **Dapagliflozin** solution and transfer in 50mL volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

Sample solution Preparation (Control Sample):

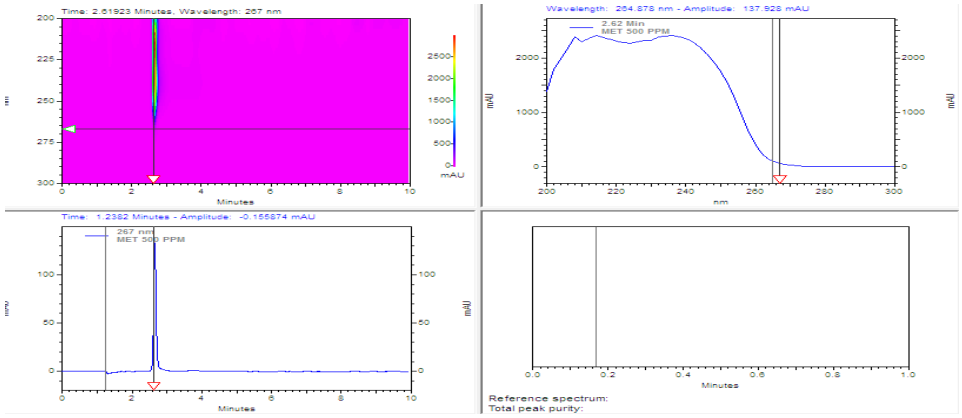
Twenty tablets each containing 100mg of **Metformin Hydrochloride** were weighed and powdered. Transfer the powder equivalent to **1000mg** of **Metformin** to 100 mL volumetric flask. Add 70 mL of diluent and sonicate for 15min. with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few mL of **Metformin** is discarded and collected as **stock sample solution**.

Twenty tablets each containing 100mg of **Sitagliptin Phosphate** were weighed and powdered. Transfer the powder equivalent to **100mg** of **Sitagliptin phosphate** to 100 mL volumetric flask. Add 70 mL of diluent and sonicate for 15min. with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few mL of **Sitagliptin phosphate** is discarded and collected as **stock sample solution**.

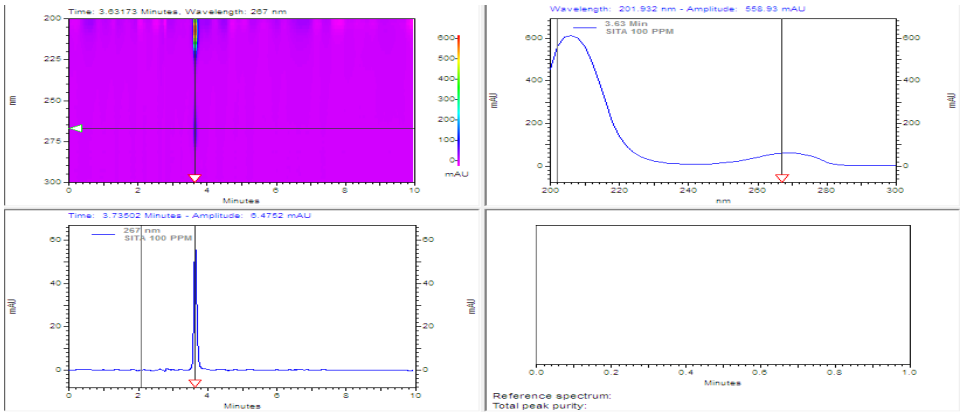
Twenty tablets each containing **10mg** of **Dapagliflozin** were weighed and powdered. Transfer the powder equivalent to **10mg** of **Dapagliflozin** to 100 mL volumetric flask. Add 70 mL of diluent and sonicate for 15min. with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few mL of **Dapagliflozin** is discarded and collected as **stock sample solution.** Pipette out standard 10mL of Dapagliflozin solution and transfer in 50mL volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

Selection of wavelength:

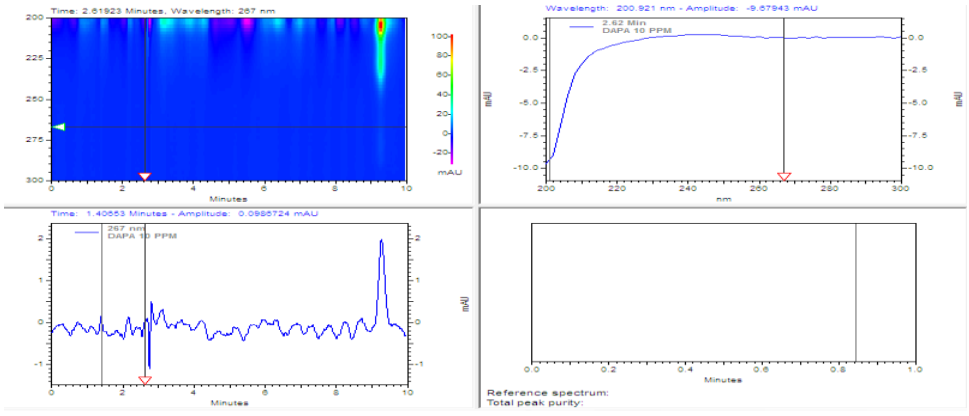
Scan the **standard solution** in HPLC between **200 nm** and **400 nm** on spectrum scan mode, the three drugs show λmax at **267 nm** for Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin.



Wavelength scan of **Metformin hydrochloride**



Wavelength scan of **Sitagliptin phosphate**



Wavelength scan of **Dapagliflozin**

Method Validation:

Specificity / Selectivity:

Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample. Typically these might include impurities, degradants, matrix, etc. The term selectivity a method can react to multiple different analytes in the sample.

Other supporting analytical procedure may make up for a particular analytical procedure's lack of specificity. The following results derives in this definition.

Identification: To confirm an analyte's identity.

Purity Tests: To ensure that each procedure used gives the details of the amount of impurities present in analyte,

From standard stock solution of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin shown in **table no- 01,** the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100- 1000** µL). The final concentration of this solution was observed in the range of **500** µg/mL for **MET**, **100** µg/mL for **SITA** and **10** µg/mL for **DAPA** respectively.

Table no. 1 Preparation of Solution of Specificity

Solution	Conc of MET in ppm	Conc of SITA in ppm	Conc of DAPA in ppm	MET Std volume µL	SITA Std volume µL	DAPA Std volume µL	Diluent µL	Final volume in µL
Standard	**500**	**100**	**10**	**500**	**100**	**50**	350	1000
Sample	**500**	**100**	**10**	**500**	**100**	**50**	350	1000

Table no. 2: Specificity data of Metformin Hydrochloride, Sitagliptin Phosphate & Dapagliflozin

SR NO	MET	SITA	DAPA

	RT	Area	Tailing Factor	RT	Area	Tailing Factor	RT	Area	Tailing Factor
1	2.700	1233716	1.26	3.653	884230	1.20	9.020	52368	1.08
2	2.700	1231111	1.19	3.640	882459	1.18	9.073	55251	1.24
3	2.720	1270333	1.17	3.660	871975	1.19	8.920	53853	0.95
4	2.713	1254380	1.23	3.647	885940	1.19	8.953	54102	0.98
5	2.707	1236170	1.21	3.667	871626	1.21	9.020	54357	1.04
6	2.720	1217802	1.13	3.660	869704	1.23	8.953	53883	0.68
MEAN	**2.710**	**1240585**	**1.20**	**3.65**	**877656**	**1.20**	**8.990**	**53969**	**1.00**
STD DEV	**0.01**	**18712**	**0.05**	**0.01**	**7305**	**0.02**	**0.06**	**938**	**0.18**
RSD	**0.34**	**1.51**	**3.82**	**0.27**	**0.83**	**1.49**	**0.64**	**1.74**	**18.57**

Precision/System Suitability:

The degree of agreement (or scatter) between a set of measurements made by repeatedly sampling the same homogeneous sample under specified conditions is expressed as the precision of an analytical method. Precision is studied in the form of repeatability, intermediate precision and reproducibility.

Study of precision is carried out using homogeneous and true samples. However, if it is difficult to obtain a homogeneous sample, artificially prepared samples or a sample solution may be used for the further study of precision.

The precision of an analytical procedure is measured as the variance, standard deviation or coefficient of variation of a set of measurements. The purpose of this is to collect data on system suitability parameters over the validation exercise. This data shall be evaluated to set the limits for the system parameters in the method.

From standard stock solution of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin shown in **table no- 01,** the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100- 1000** µL). The final concentration of this solution was observed in the range of **500** µg/mL for **MET**, **100** µg/mL for **SITA** and **10** µg/mL for **DAPA** respectively.

Table no. 3: Preparation of Solution of Precision/System Suitability

Solution	Conc of MET in ppm	Conc of SITA in ppm	Conc of DAPA in ppm	MET Std volume µL	SITA Std volume µL	DAPA Std volume µL	Diluent µL	Final volume in µL
Standard	**500**	**100**	**10**	**500**	**100**	**50**	350	1000
Sample	**500**	**100**	**10**	**500**	**100**	**50**	350	1000

Table no. 4: Precision/System Suitability data of Metformin Hydrochloride, Sitagliptin Phosphate & Dapagliflozin

Sr. No.	MET			SITA			DAPA		
	RT	Area	Tailing Factor	RT	Area	Tailing Factor	RT	Area	Tailing Factor
1	2.700	1337009	1.22	3.64	845333	1.16	9.233	52081	1.20
2	2.700	1340284	1.16	3.640	853592	1.25	9.227	53138	1.03
3	2.693	1309938	1.13	3.647	860374	1.20	9.247	53510	1.11
4	2.700	1345453	1.13	3.640	858660	1.17	9.113	52818	1.12
5	2.693	1327694	1.20	3.613	850799	1.26	9.193	53834	1.13
6	2.687	1312236	1.21	3.607	849793	1.19	9.133	52315	1.08
MEAN	**2.696**	**1328769**	**1.18**	**3.63**	**853092**	**1.21**	**9.191**	**52949**	**1.11**
STD DEV	**0.01**	**14885.91**	**0.04**	**0.02**	**5668**	**0.04**	**0.056**	**679**	**0.06**
RSD	**0.20**	**1.12**	**3.44**	**0.46**	**0.66**	**3.43**	**0.609**	**1.28**	**5.07**

Linearity:

The linearity of an analytical method is its ability to give test results that are directly or indirectly proportional to the concentration of the substance in the samples within a given range, as determined by a well-defined mathematical transformation.

From standard stock solution of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin shown in **table no- 01,** the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100- 1000** µL). The final concentration of this solution was observed in the range of **250–750** µg/mL for **MET**, **50–150** µg/mL for **SITA** and **5–15** µg/mL for **DAPA** respectively. To obtain the calibration curve and correlation coefficients, the calibration curves with observed peak areas against concentration are plotted. For the confirmation of good linearity of the method, characteristics parameters for regression equation (y = mx+c) of the method and these parameter were used. The results are shown in **Table no 2, 3 and 4** and **Graph 1, 2 and 3.**

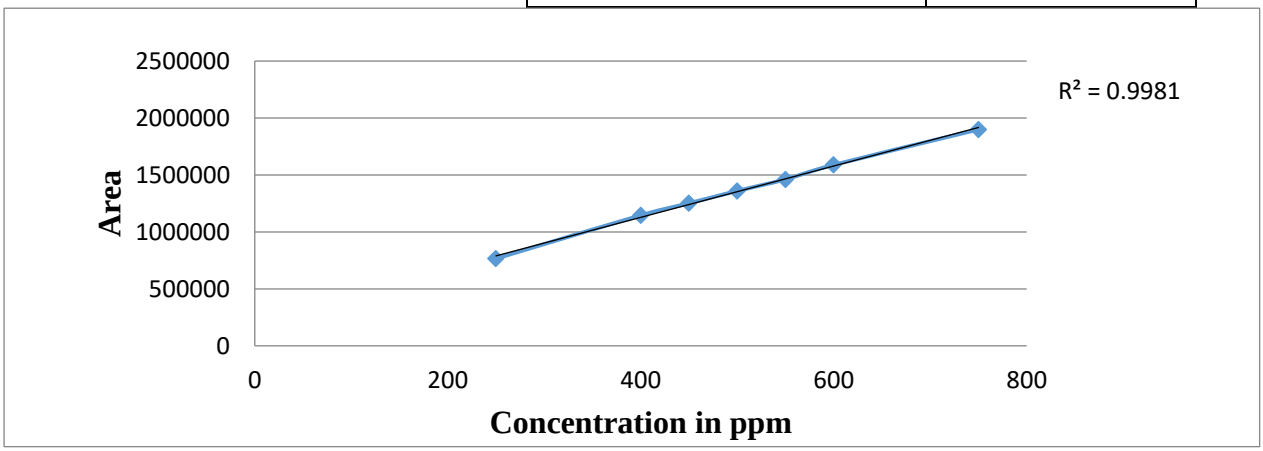
Table no. 5: Preparation of Solution of Linearity

Sr. No.	Solution in %	Conc of MET in ppm	Conc of SITA in ppm	Conc of DAPA in ppm	MET Std volume µL	SITA Std volume µL	DAPA Std volume µL	Diluent µL	Final volume in µL
1	**50%**	**250**	**50**	**5**	**250**	**50**	**25**	675	1000
2	**80%**	**400**	**80**	**8**	**400**	**80**	**40**	480	1000
3	**90%**	**450**	**90**	**9**	**450**	**90**	**45**	415	1000
4	**100%**	**500**	**100**	**10**	**500**	**100**	**50**	350	1000
5	**110%**	**550**	**110**	**11**	**550**	**110**	**55**	285	1000
6	**120%**	**600**	**120**	**12**	**600**	**120**	**60**	220	1000
7	**150%**	**750**	**150**	**15**	**750**	**150**	**75**	25	1000

Table no. 6: Linearity data of Metformin Hydrochloride

Sr. No.	Solution %	Conc in ppm	Area
1	50%	250	765829
2	80%	400	1145477
3	90%	450	1251418
4	100%	500	1359565

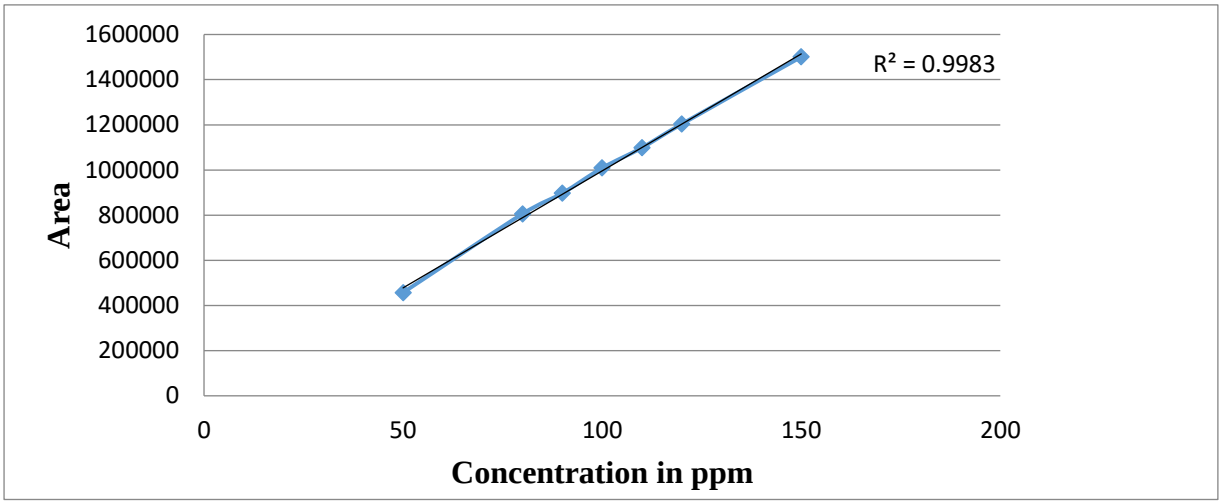
5	110%	550	1461441
6	120%	600	1588382
7	150%	750	1899415
		Correlation Coefficient	**0.9990**



Graph 1: Calibration curve of Metformin

Table no. 7: Linearity data of Sitagliptin Phosphate

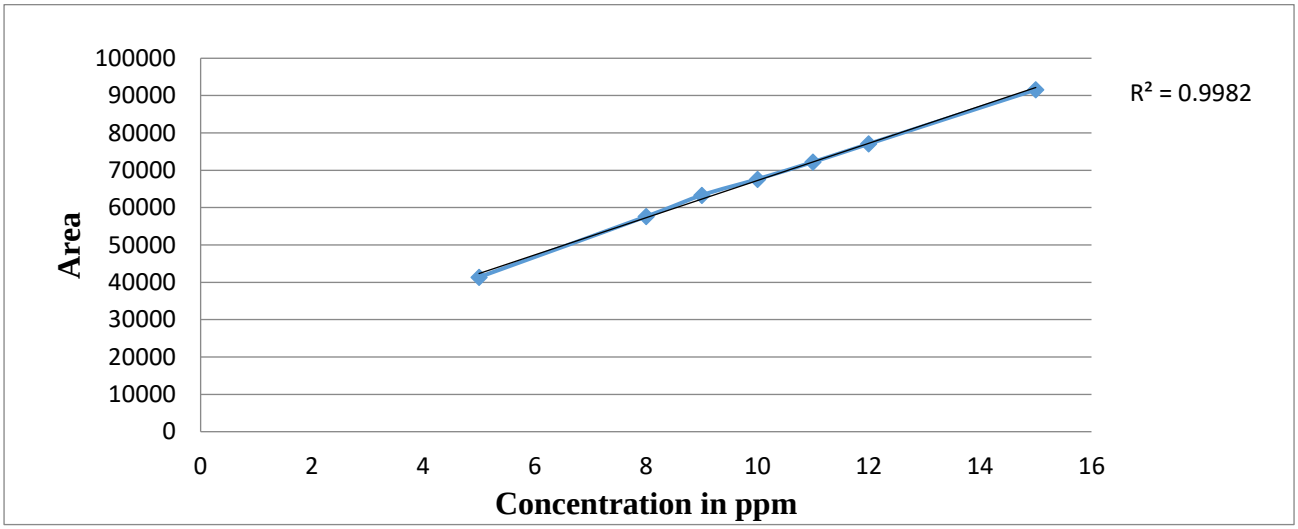
Sr. No.	Solution %	Conc in ppm	Area
1	50%	50	**457176**
2	80%	80	**805692**
3	90%	90	**896992**
4	100%	100	**1008887**
5	110%	110	**1098604**
6	120%	120	**1203707**
7	150%	150	**1500744**
		Correlation Coefficient	**0.9992**



Graph 2: Calibration curve of Sitagliptin

Table no 8: Linearity data of Dapagliflozin

Sr No	Solution %	Conc in ipm	Area
1	50%	5	**41377**
2	80%	8	**57611**
3	90%	9	**63329**
4	100%	10	**67623**
5	110%	11	**72219**
6	120%	12	**77080**
7	150%	15	**91595**
		Correlation Coefficient	**0.9991**



Graph 3: Calibration curve of Dapagliflozin

Accuracy:

The accuracy of an analytical method refers to how closely the test results produced by that method match with the true value. Accuracy can be measured by analyzing known amounts of analyte with a present recovery.

From standard stock solution of MET, SITA & DAPA shown in **table no - 04**, the different concentration of solution is prepared by using **calibrated micropipette (10- 100** µL & **100-1000** µL pipette). The accuracy is determined for MET, SITA and DAPA (equivalent to **500 mg of MET** and **100 mg of SITA** and **10 mg of DAPA**) (50%, 100%, and 150% of solutions respectively) to a quantity equal to the average weight of marketed tablets. The combinations of drugs were examined in triplicate. The percentage recovery of added drugs is used to evaluate accuracy. The results are shown in Table no **5, 6, 7 & 8**.

Table No. 9: Preparation of Solution of Accuracy

Sr. No.	Solution %	Conc in ppm	Met Std µL	Sita Std µL	Dapa Std µL	Diluent µL	Final volume in µL
1	**100% STD**	500	**500**	**100**	**50**	350	1000
2	50%	250	**250**	**50**	**25**	675	1000
3	100%	500	**500**	**100**	**50**	350	1000
4	150%	750	**750**	**150**	**75**	25	1000

Table no 10: Accuracy data of Metformin Hydrochloride

MET		Wt in mg	Conc.	Ppm
Std Wt.		100.0	**Std conc.**	500
Sample Wt.		100.0	50 % Sample conc.	250
			100 % Sample conc.	500
			150 % Sample conc.	750
No of Inj.	**Std Area**	**50% Sample Area**	**100% Sample Area**	**150% Sample Area**
Inj-1	**1299730**	663109	1314965	1983027
Inj-2	**1343440**	652721	1328683	2001547
Inj-3	**1319137**	661049	1315256	1987122

Inj-4	**1309347**			
Inj-5	**1331086**			
Mean	**1320548**	**658960**	**1319635**	**1990565.333**
Std. Dev.	**17291.2**	**5500.1**	**7837.4**	**9728.3**
RSD	**1.309**	**0.835**	**0.594**	**0.489**
%		**99.80**	**99.93**	**100.49**
	Formula	$\frac{\text{Sample Area x Standard Conc.}}{\text{Standard Area x Sample Conc.}}$ **X 100**		

Table no. 11: Accuracy data of Sitagliptin Phosphate

SITA		**Wt in mg**	**Conc.**	**ppm**
std wt.		100.0	**Std conc.**	100
Sample wt.		100.0	50 % Sample conc.	50
			100 % Sample conc.	100
			150 % Sample conc.	150
No Of Inj.	**Std Area**	**50% Sample Area**	**100% Sample Area**	**150% Sample Area**
Inj-1	**1008714**	503368	1018730	1515880
Inj-2	**1028747**	509688	1022687	1529152
Inj-3	**1011193**	501251	1005785	1507322
Inj-4	**1020962**			
Inj-5	**1016318**			
Mean	**1017187**	**504769**	**1015734**	**1517451**
Std. Dev.	**8005.6**	**4389.5**	**8840.3**	**10999.5**
RSD	**0.787**	**0.870**	**0.870**	**0.725**
%		**99.25**	**99.86**	**99.45**
	Formula	$\frac{\text{Sample Area x Standard Conc.}}{\text{Standard Area x Sample Conc.}}$ **X 100**		

Table no. 12: Accuracy data of Dapagliflozin

DAPA		Wt in mg	Conc.	ppm
Std Wt.		100.0	Std conc.	10
Sample Wt.		100.0	50 % Sample conc.	5
			100 % Sample conc.	10
			150 % Sample conc.	15
No of Inj.	**Std Area**	**50% Sample Area**	**100% Sample Area**	**150% Sample Area**
Inj-1	**53863**	26755	54032	81987
Inj-2	**54763**	26977	53692	81050
Inj-3	**53510**	26910	54798	81625
Inj-4	**54282**			
Inj-5	**53684**			
Mean	**54020**	**26881**	**54174**	**81554**
Std. Dev.	**504.6**	**113.9**	**566.5**	**472.5**
RSD	**0.934**	**0.424**	**1.046**	**0.579**
%		**99.52**	**100.28**	**100.65**
	Formula	$\frac{\textbf{Sample Area x Standard Conc.}}{\textbf{Standard Area x Sample Conc.}}$ **X 100**		

Robustness:

The sample solution of Metformin hydrochloride, Sitagliptin phosphate and Dapagliflozin are prepared having concentration of **500 mg/mL of MET** and **100 mg/mL of SITA** and **10 mg/mL of DAPA** respectively and analyzed using different chromatographic condition as below and to show the system is stable within the chromatographic conditions of the proposed method.

a) Change in flow rate (0.8 mL/min & 1.2 mL/min)

b) Change in temperature (27°C & 33°C)

i. Change in flow: Standard solution and sample solution for five replicate injections are injected at **different flow rates**. The results obtained are shown in below mentioned

chromatograms. The results are shown in the **table 10 & 11**.

ii. Change in temperature: Standard solution and sample solution for five replicate injections are injected at **different temperatures**. The results obtained are shown in below mentioned chromatograms. The results are shown in the **table 12 &13**.

Table no. 13: Preparation of Solution of MET, SITA & DAPA

Sr No	Solution %	Met Std in µL	Sita Std in µL	Dapa Std in µL	Diluent in µL	Final volume in µL
1	**100% STD**	**500**	**100**	**50**	350	1000
2	**100% SPL**	**500**	**100**	**50**	350	1000

Table no. 14: Change in flow rate 0.8 mL/min of MET, SITA & DAPA

	MET		SITA		DAPA	
No of Inj.	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**
Inj-1	1667261	1670911	1254417	1246694	89779	89071
Inj-2	1676301	1667991	1206150	1220001	87789	88192
Inj-3	1664936	1678200	1241210	1223835	87584	87892
Inj-4	1620249		1239178		88328	
Inj-5	1673170		1261129		87128	
Mean	**1660383.4**	**1672367**	**1240417**	**1230177**	**88122**	**88385**
STDEV	**22889.2**	**5258.0**	**21221.3**	**14432.3**	**1021.8**	**612.7**
RSD	**1.379**	**0.314**	**1.711**	**1.173**	**1.160**	**0.693**
%		**100.72**		**99.17**		**100.30**

Table no. 15: Change in flow rate 1.2 mL/min of MET, SITA & DAPA

	MET		SITA		DAPA	
No of Inj.	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**
Inj-1	1108066	1104146	836092	846424	53119	53969
Inj-2	1103931	1077999	852147	851628	51763	53367
Inj-3	1113531	1104744	858796	832677	53925	53967
Inj-4	1087327		830056		53625	
Inj-5	1090064		842123		53275	
Mean	**1100583.8**	**1095630**	**843843**	**843576**	**53141**	**53768**
STDEV	**11415.2**	**15271.5**	**11679.8**	**9791.2**	**831.6**	**347.0**
RSD	**1.037**	**1.394**	**1.384**	**1.161**	**1.565**	**0.645**
%		**99.55**		**99.97**		**101.18**

Table no. 16: **Change in Temperature 27°C of MET, SITA & DAPA**

	MET		SITA		DAPA	
No of Inj.	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**
Inj-1	1502784	1536890	1102497	1131972	71850	71077
Inj-2	1529195	1544633	1125224	1129754	71790	70496
Inj-3	1546779	1545242	1110627	1112310	71513	71631
Inj-4	1568028		1102167		70935	
Inj-5	1506644		1114370		71053	
Mean	**1530686**	**1542255**	**1110977**	**1124679**	**71428**	**71068**
STDEV	**27441.6**	**4656.2**	**9541.2**	**10768.8**	**418.3**	**567.6**
RSD	**1.793**	**0.302**	**0.859**	**0.958**	**0.586**	**0.799**
%		**100.76**		**101.23**		**99.50**

Table no. 17: **Change in Temperature 33°C of MET, SITA & DAPA**

	MET		SITA		DAPA	
No of Inj.	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**	**Std Area**	**Spl Area**
Inj-1	1551252	1551936	1149681	1147692	73267	73704

Inj-2	1520088	1547977	1103210	1144463	72586	72488
Inj-3	1509497	1511252	1147912	1149681	72501	72267
Inj-4	1506111		1124712		72426	
Inj-5	1488738		1146362		74309	
Mean	**1515137.2**	**1537055**	**1134375**	**1147279**	**73018**	**72820**
STDEV	**23124.6**	**22433.6**	**20160.9**	**2633.4**	**795.8**	**773.8**
RSD	**1.526**	**1.460**	**1.777**	**0.230**	**1.090**	**1.063**
%		**101.45**		**101.14**		**99.73**

Results and Discussions:

Specificity/ Selectivity:

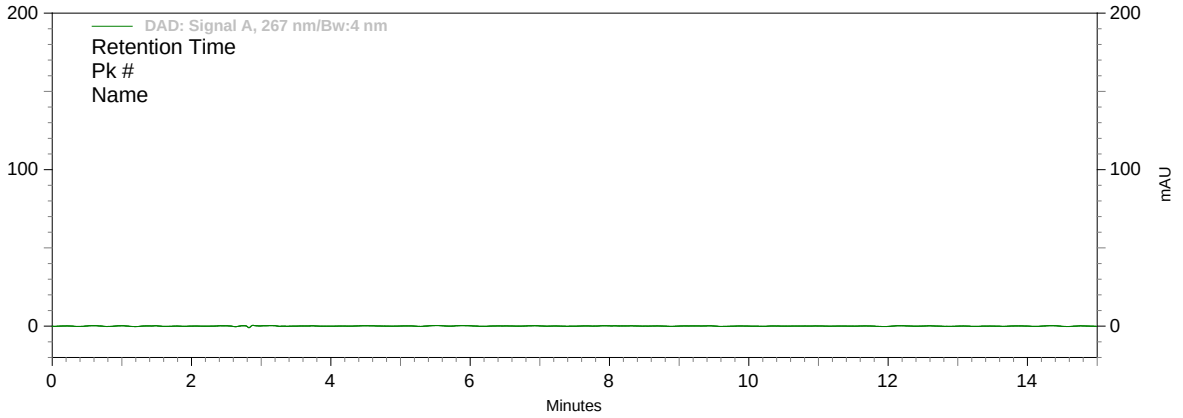
From Table no. 2: Specificity/Selectivity Results

Name of Drug	RSD (RT)	RSD (Area)	Tailing Factor (Mean)
MET	0.34	1.51	1.20
SITA	0.27	0.83	1.20
DAPA	0.64	1.74	1.00

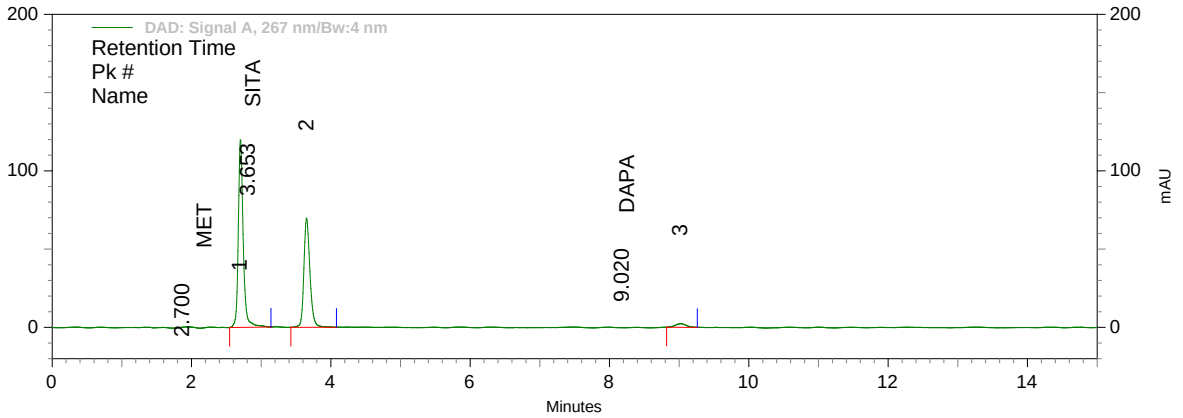
The specificity and selectivity of this proposed method could be explained through the standard addition method where satisfactory recoveries and standard deviation were obtained in the presence of different pharmaceutical dosage forms. The chromatograms of drugs in various tablet formulations show that excipients do not interfere with the detection wavelength in the proposed method.

As from above data the RSD observed are within the limit as per required criteria standards.

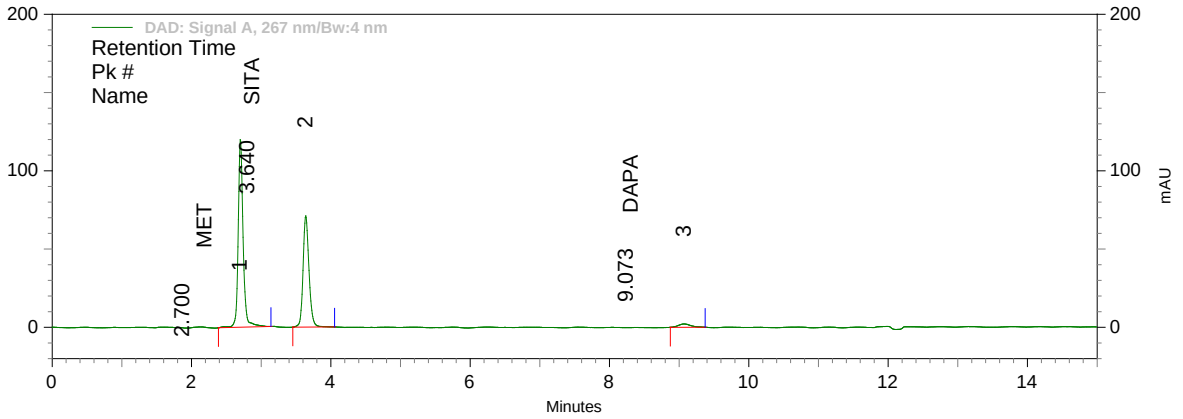
The representative chromatogram obtained for blank in **specificity/selectivity** of MET, SITA & DAPA is shown in **figure 1**.



The representative chromatogram obtained for **standard solution** shown in **specificity/selectivity** of MET, SITA & DAPA in **figure 2**



The representative chromatogram obtained for **sample solution** shown in **specificity/selectivity** of MET, SITA & DAPA in **figure 3**.



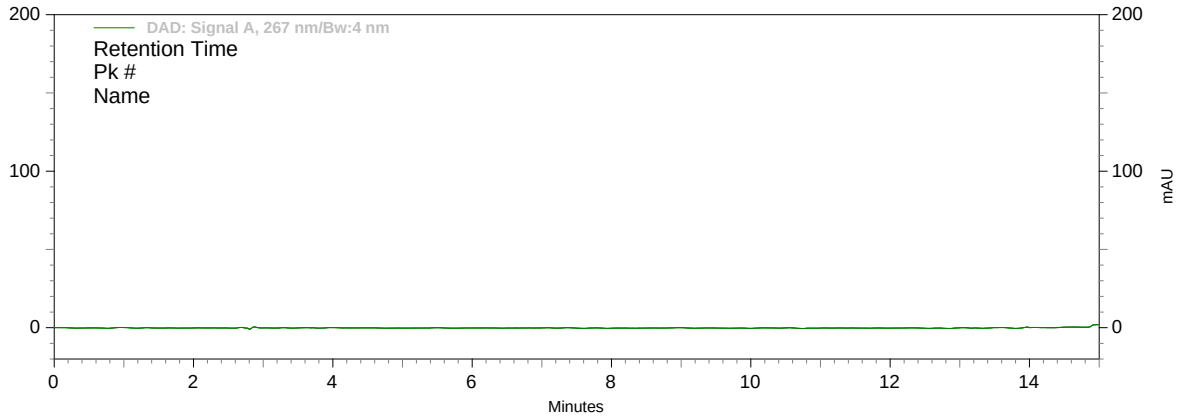
Precision/System Suitability:

From Table no. 4: Results

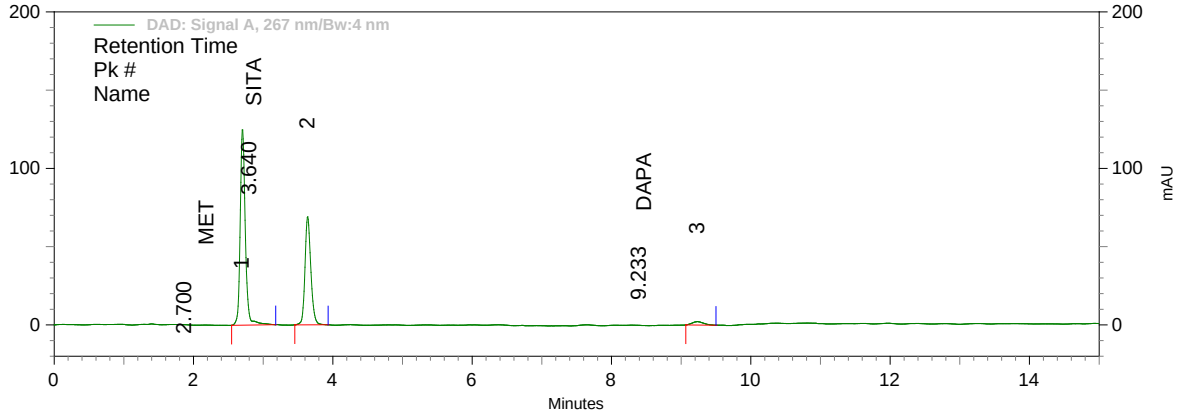
Name of Drug	RSD (RT)	RSD (Area)	Tailing Factor (Mean)
MET	0.20	1.12	1.18
SITA	0.46	0.66	1.21
DAPA	0.61	1.28	1.11

From above data the RSD observed are within the limit as per required criteria standards.

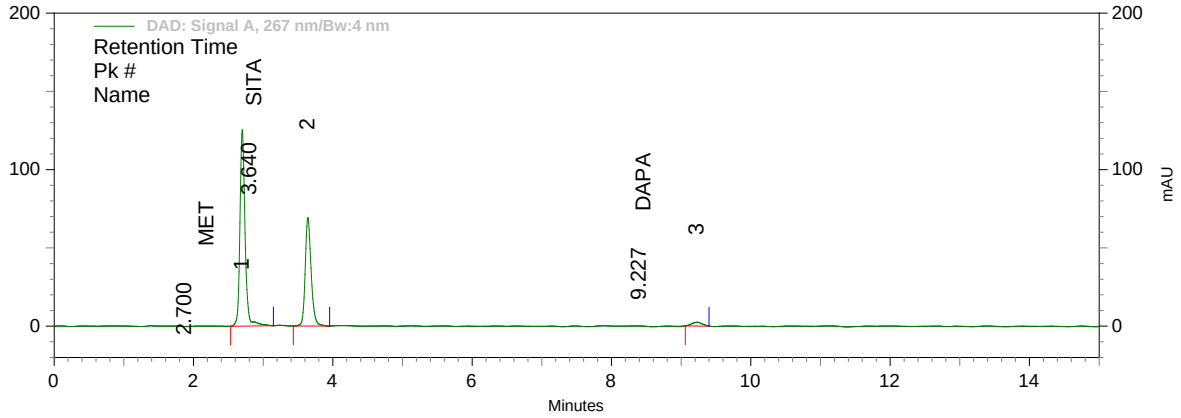
The representative chromatogram obtained for blank in **Precision/System Suitability** of MET, SITA & DAPA is shown in **figure 4.**



The representative chromatogram obtained for **standard solution** shown in **Precision/System Suitability** of MET, SITA & DAPA in **figure 5.**



The representative chromatogram obtained for **sample solution** shown in **Precision/System Suitability** of MET, SITA & DAPA in **figure 6**.



Linearity:

From Table no. 5, 6 and 7: Results

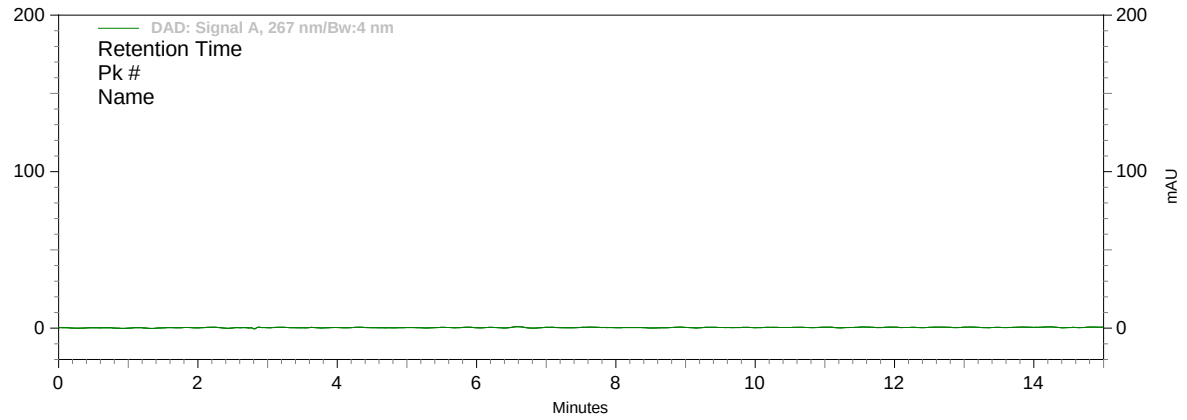
The retention time was found to be **2.673** minutes for **Metformin hydrochloride, 3.560** minutes for **Sitagliptin Phosphate** and **9.060** for **Dapagliflozin** respectively. The proposed method was found to be having **linearity** in the concentration range of **250-750** µg/mL for **MET** (**R^2= 0.9990**), **50-150** µg/mL for **SITA** (**R^2= 0.9992**) and **5-15** µg/mL for **DAPA** (**R^2= 0.9991**) respectively.

Table no. 14: Acceptance criteria.

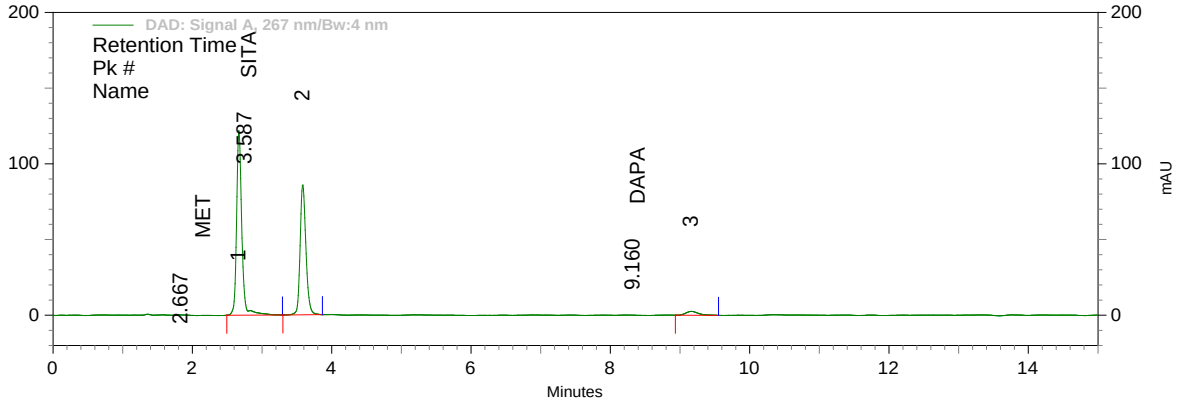
Parameters	Observed Results	Acceptance Criteria
Correlation Coefficient of MET	0.9990	NLT 0.99
Correlation Coefficient of SITA	0.9992	NLT 0.99
Correlation Coefficient of DAPA	0.9991	NLT 0.99

It was observed that the proposed method was found to be having **linearity** in the concentration range of **250–750** µg/mL for **MET**, **50–150** µg/mL for **SITA** and **5–15** µg/mL for **DAPA** respectively.

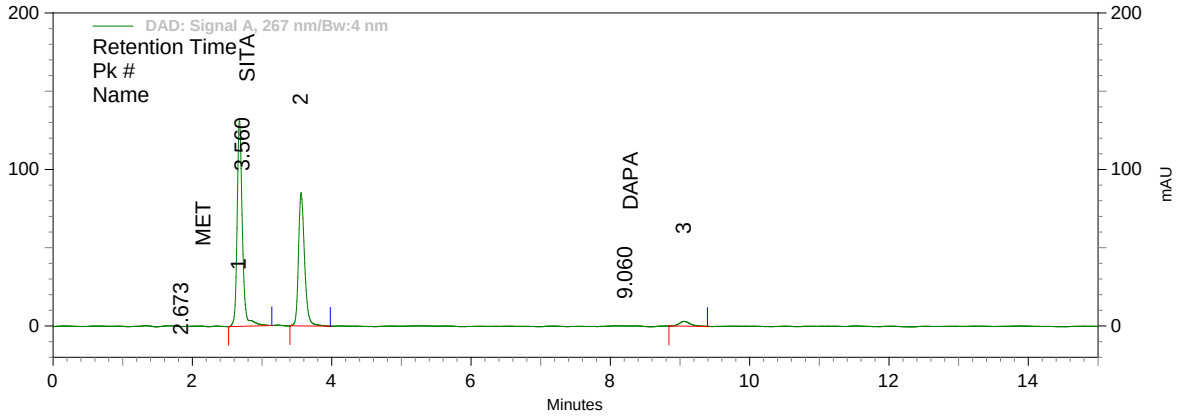
The representative chromatogram obtained for blank in **linearity** of MET, SITA & DAPA is shown in **figure 1**.



The representative chromatogram obtained for **standard solution** shown in **linearity** of MET, SITA & DAPA in **figure 2**.



The representative chromatogram obtained for **sample solution** shown in **linearity** of MET, SITA & DAPA in **figure 3**.



Accuracy:

Table No. 8, 9 and 10: Results

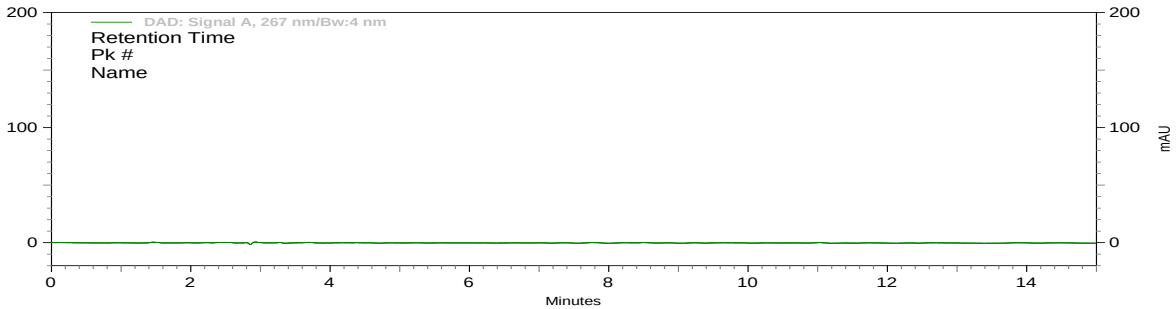
The mean % **recoveries** obtained were found to be **98.00-102.00%** for **MET ,98.00-102.00%** for **SITA** and **98.00 -102.00%** for **DAPA** respectively.

Table no. 15: Acceptance criteria.

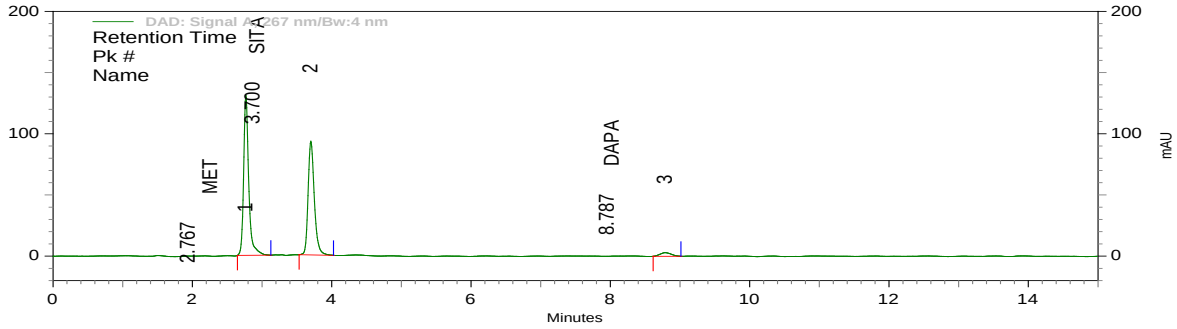
Parameters	Observed Results	Acceptance Criteria
Accuracy of MET	50% - **99.80%** 100% - **99.93%** 150% - **100.49%**	Between 98% to 102%
Accuracy of SITA	50% - **99.25%** 100% - **99.86%** 150% - **99.45%**	Between 98% to 102%
Accuracy of DAPA	50% - **99.52%** 100% - **100.28%** 150% - **100.65%**	Between 98% to 102%

It was observed that the mean % **recoveries** obtained were found to be **98.00-102.00%** for **MET** , **98.00-102.00%** for **SITA** and **98.00 -102.00%** for **DAPA** respectively.

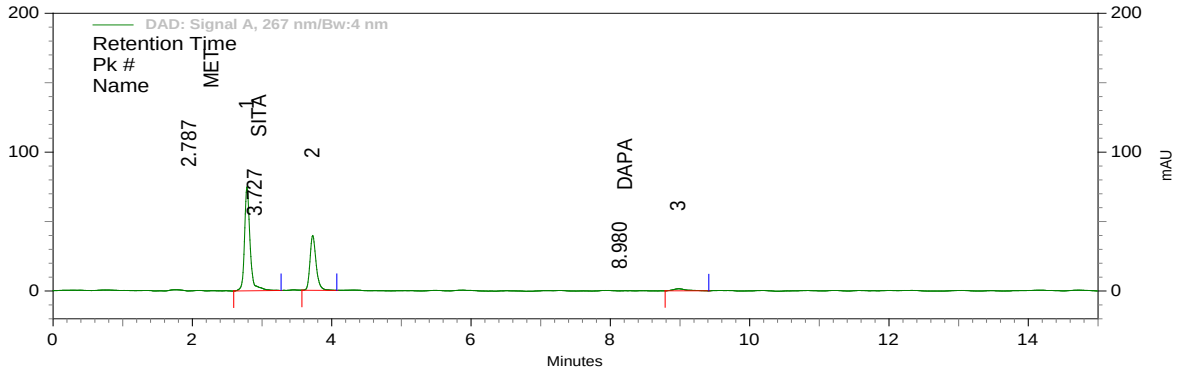
The representative chromatogram obtained for **blank** in **accuracy** of MET, SITA & DAPA shown in **figure 4**.



The representative chromatogram obtained for **standard solution** shown in **accuracy** of MET, SITA & DAPA in **figure 5**.



The representative chromatogram obtained for **sample solution** shown in **accuracy** of MET, SITA & DAPA in **figure 6.**



Robustness:

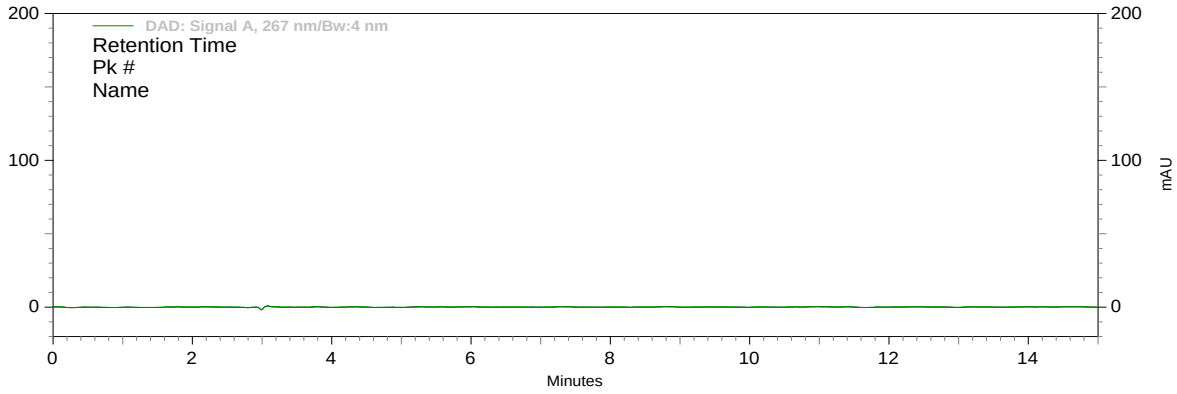
Table No. 13 to 17: Results

The system suitability parameters and % RSD of Standard & Assay of Samples are estimated. The values are given below.

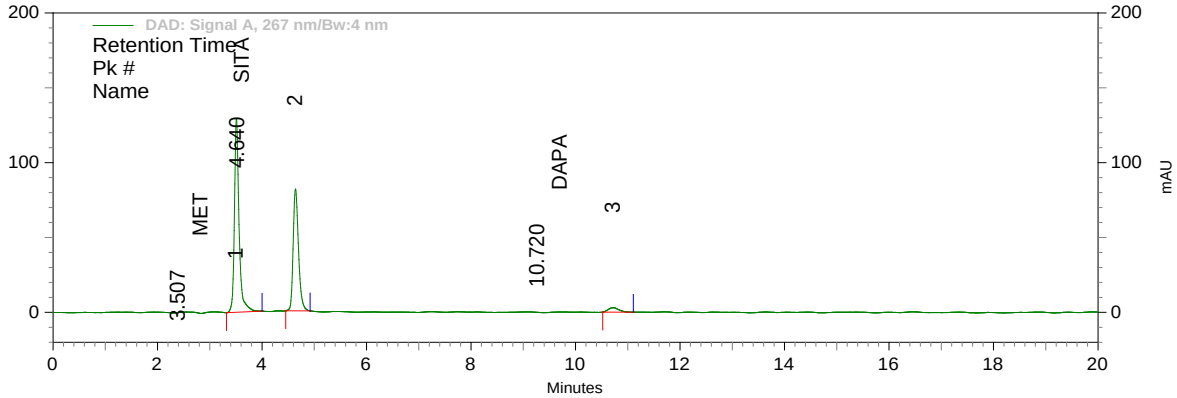
Sr. No	Change parameter	Name of Drug	% RSD of STD	Assay of SPL
1	Flow Rate **0.8** mL/min	MET	1.793	100.76
		SITA	0.859	101.23
		DATA	0.586	99.50
2	Flow Rate **1.2** mL/min	MET	1.526	101.45
		SITA	1.777	101.14
		DATA	1.090	99.736
3	Column Temperature **27°C**	MET	1.379	100.72
		SITA	1.711	99.17
		DATA	1.160	100.30
4	Column Temperature **33°C**	MET	1.037	99.55
		SITA	1.384	99.97
		DATA	1.565	101.18

By varying the **flow rate & temperature**, it was observed that there are no major changes are found in the chromatogram. It is concluded that the method developed was robust at the given parameters which shows within the acceptance criteria and the method exhibit good system suitability under the given set of conditions.

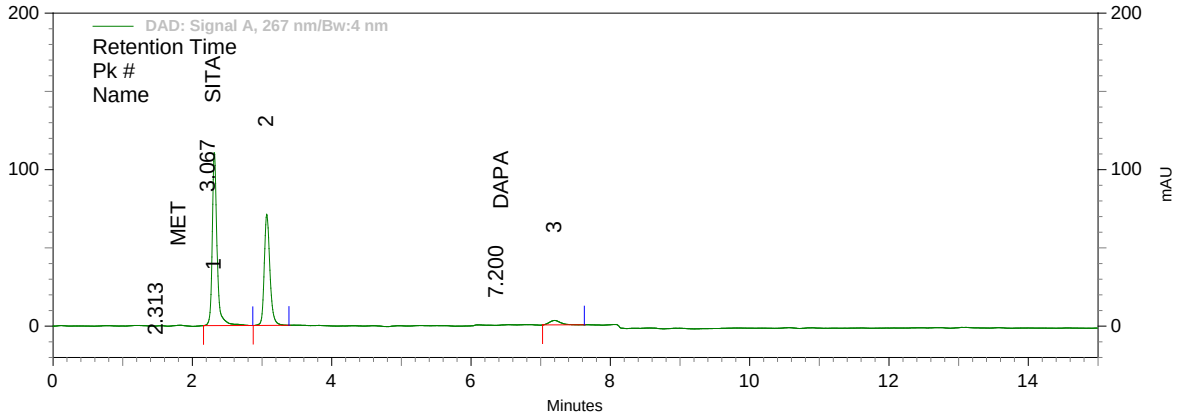
The representative chromatogram obtained for **blank** in **Robustness at 0.8 mL/min flow rate** of MET, SITA & DAPA shown in **figure 7**.



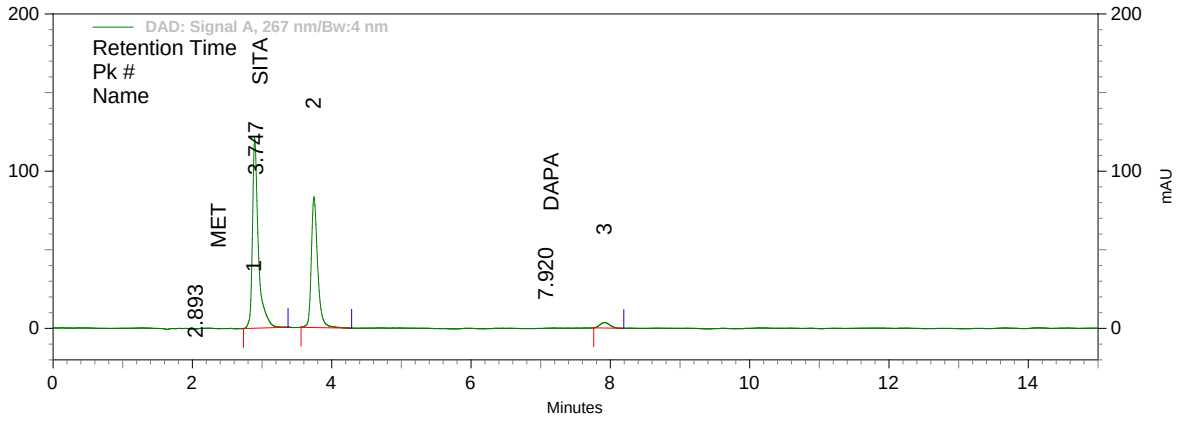
The representative chromatogram obtained for **STD** in **Robustness at 0.8 mL/min flow rate** of MET, SITA & DAPA shown in **figure 8**.



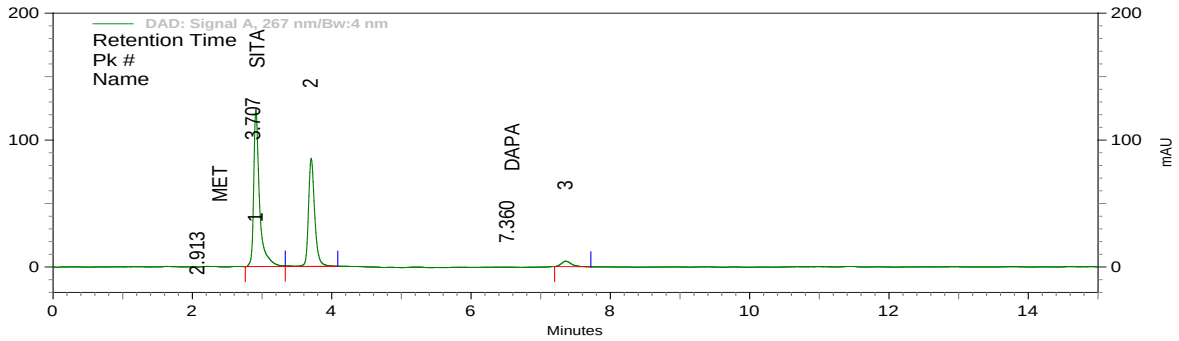
The representative chromatogram obtained for **STD** in **Robustness at 1.2 mL/min flow rate** of MET, SITA & DAPA shown in **figure 9**.



The representative chromatogram obtained for **STD** in **Robustness at 27°C** of MET, SITA & DAPA shown in **figure 10**.



The representative chromatogram obtained for **STD** in **Robustness at 33°C** of MET, SITA & DAPA shown in **figure 11**.



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