Stability Indicating Rp-Uplc Method Development And Validation For The Simultaneous Estimation Of Metformin Hydrochloride, Saxagliptin And Dapagliflozin In Their Bulk And Pharmaceutical Dosage Forms

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ABSTRACT
A simple, rapid and sensitive method is developed for the simultaneous estimation of Metformin HCl, Saxagliptin and Dapagliflozin in their bulk and pharmaceutical dosage forms by RP-UPLC. The solubility studies of the drugs were carried out at 25^0C using distilled water, methanol, acetonitrile and buffers as solvents. The analysis of drugs is carried out using Acquity UPLC BEH C18 column (10mm × 2.1mm ID, 1.7µm) at 280nm wavelength with mobile phase composition of 0.02M phosphate buffer pf pH 4.5 and acetonitrile in the ratio of 70:30. The retention time for Metformin HCl, Saxagliptin and Dapagliflozin is found to be 2.601, 4.329 and 5.238 respectively. The developed method is validated for various parameters like linearity, accuracy, precision, robustness, specificity, and system suitability as per ICH guidelines. The stability of the drugs is assessed by various stress conditions like acid, alkali, peroxide, photolytic and thermal degradation. All the results were found to be within the acceptance limits. Hence the present method can be conveniently adopted for the estimation of Metformin HCl, Saxagliptin and Dapagliflozin.

Keywords: Metformin HCl, Saxagliptin, Dapagliflozin, RP-UPLC, retention time and ICH guidelines.

INTRODUCTION

Diabetes mellitus is one of the most common medical conditions globally. The number of people with diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity.[1] A multi-target anti-diabetic drug combination composed of Metformin, Saxagliptin and Dapagliflozin recently approved for the Type 2 diabetes mellitus.[2] Metformin Hydrochloride is chemically known as (S)-Isopropyl 2-((S)-(((2R,3R,4R,5R)-5- (2,4-dioxo -3,4-dihydropyrimidin-1 (2H)-yl)-4-fluoro-3-hydroxy -4-methyltetrahydrofuran -2-yl) methoxy)- (phenoxy) phosphorylamino) propanoate.[3] Saxagliptin is also oral antidiabetic drug and is belongs to new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Chemical name is (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile.[4] Dapagliflozin (DAPA) is chemically described as (1s)-1,5-anhydro-1-C-[4-chloro-3-[(4-ethoxy phenyl)methyl]phenyl]-D-glucitol. It belongs to a new class of oral antidiabetic drugs called sodium glucose cotransporter 2 (SGLT2) inhibitors.[5]

Metformin, Saxagliptin, and Dapagliflozin is an antidiabetic medication used as an adjunct to diet and exercise to improve glycemic control in adults with Type 2 diabetes [1]. It is taken orally. The most common side effects include nose and throat infections, hypoglycemia and effects on the gut such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite. The fixed-dose combination was approved for medical use in the United States in May 2019 and in the European Union in November 2019.[6-8]

The literature survey reveals that only HPLC studies were carried out so far for the estimation of selected drugs hence in the present study an attempt is made to analyze the selected compounds by UPLC which is a relatively new technique with high sensitivity and resolution.
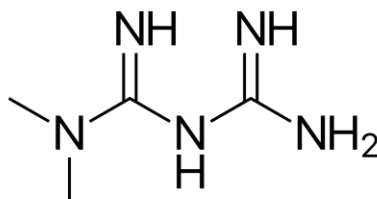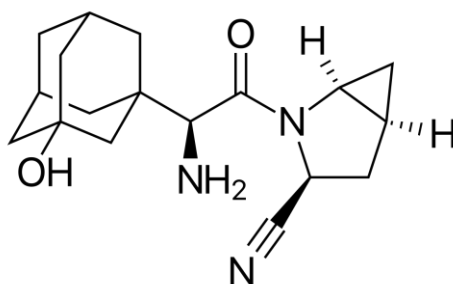
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Figure-1: Structure of Metformin
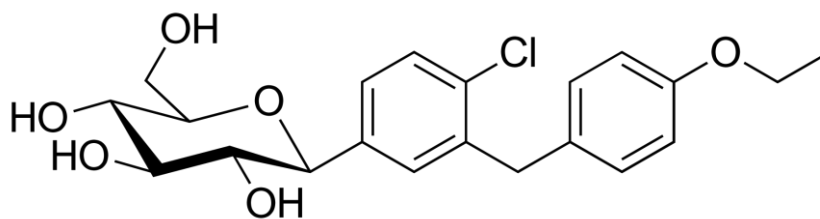
Figure-2: Structure of Saxagliptin

Figure-3: Structure of Dapagliflozin

EXPERIMENTAL

Chemicals and reagents:
Metformin hydrochloride, Saxagliptin and Dapagliflozin pure drugs (API) were received from Jigs Chemical Limited, Ahmedabad, India. Qternmet XR dosage form (Saxagliptin 5mg / Metformin 1000mg / Dapagliflozin 10mg) were purchased from local market. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer and Ortho-phosphoric acid were purchased from Rankem chemicals. UPLC of Agilent 1290 Infinity II make with DAD (Diode Array Detector), Shimadzu/AY220 Analytical balance, ultrasonicate water bath from PCI Analytics-Mumbai and pH meter of Digisun electronics/2001 were used in the study.

Chromatographic conditions:
The determination of Metformin hydrochloride, Saxagliptin and Dapagliflozin were achieved with the help of stationary phase as Acuity UPLC BEH C18 (100 × 2.1 mm ID, 1.7 μm) column with isocratic elution using 0.02M phosphate buffer (pH 4.5) and acetonitrile in the ratio of 70:30 as solvent. 0.3 ml/min is the flow rate set with a run time of 8 min. The wavelength for detection is set at 280 nm with the temperature of the column is set at 25°C. 10 µL is the injection volume used for the determination.

Preparation of standard solution of Metformin hydrochloride:
10mg of Metformin HCl is weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then made the volume up to the mark with methanol and prepared 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

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Preparation of standard solution of Saxagliptin:
10mg of Saxagliptin is weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then made the volume up to the mark with methanol and prepared 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard solution of Dapagliflozin:
10mg of Dapagliflozin is weighed and transferred in to 100 ml volumetric flask and dissolved in methanol and then made the volume up to the mark with methanol and prepared 10 µg/ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution:
Weighed accurately 100mg of Metformin HCl, 50mg of Dapagliflozin and 25mg of in 100 ml of volumetric flask and dissolved in 70ml of mobile phase and made the volume with mobile phase. From above stock solution 100µg/ml of Metformin HCl, 50µg/mL of Dapagliflozin and 25µg/mL of Saxagliptin were prepared by diluting 5ml to 50ml with mobile phase respectively.

RESULTS AND DISCUSSION

In the present work, a novel RP-UPLC method is developed for the simultaneous estimation of Metformin HCl, Saxagliptin and Dapagliflozin. The method employed uses fewer organic solvents and maintain a minimal mobile phase without the addition of buffer salts or pH correction. Metformin HCl's retention time is found to be 2.601 min, while Saxagliptin's is 4.329 min and 5.238 for Dapagliflozin. The developed method is validated using ICH Q2 (R1) guidelines to see if it was accurate for analyzing Metformin HCl, Saxagliptin and Dapagliflozin.
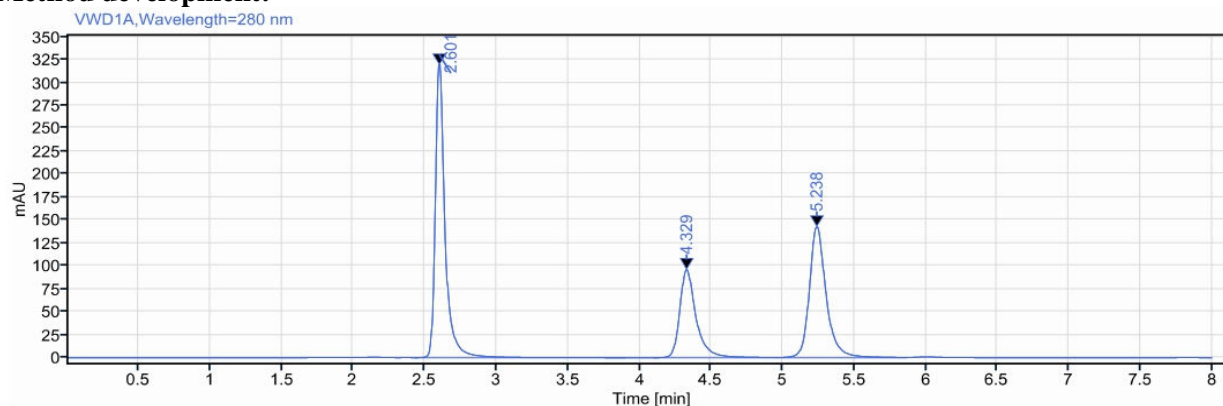
Method development:

Figure-4: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin

Method validation:
Linearity:

*STABILITY INDICATING RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE
SIMULTANEOUS ESTIMATION OF METFORMINHYDROCHLORIDE, SAXAGLIPTIN AND
DAPAGLIFLOZIN IN THEIR BULK AND PHARMACEUTICAL DOSAGE FORMS*
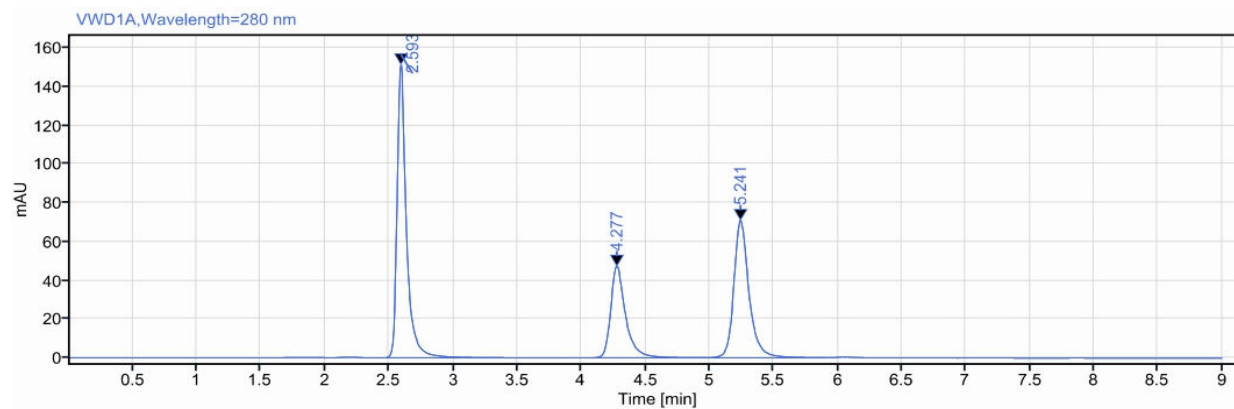
Section A-Research paper

Figure-5: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin (Preparation-1)
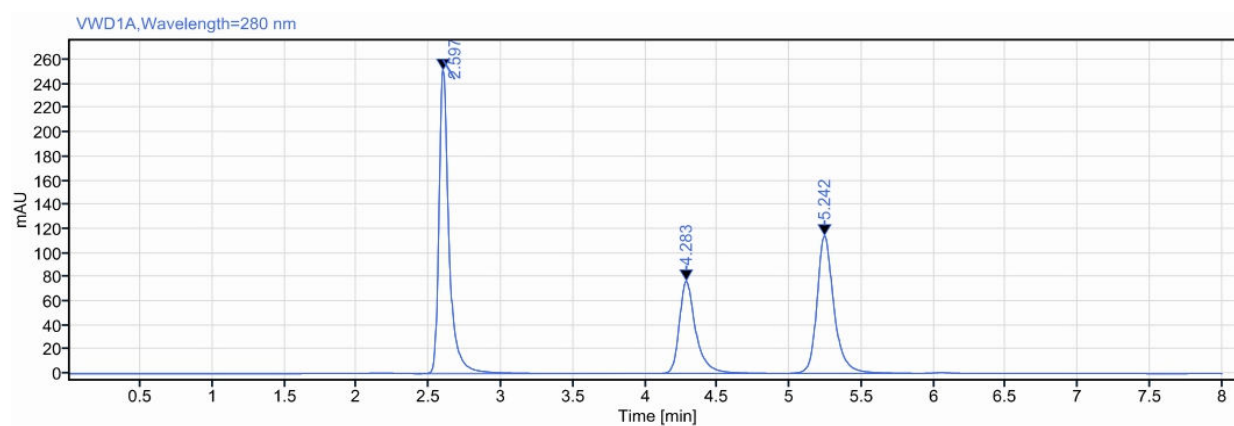

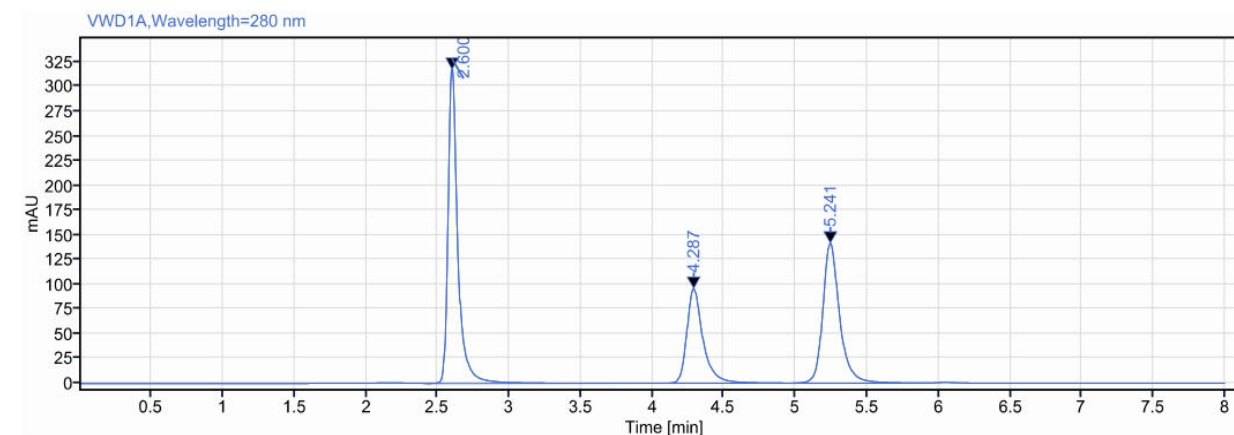Figure-6: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin (Preparation-2)

Figure-7: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin (Preparation-3)

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*STABILITY INDICATING RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE
SIMULTANEOUS ESTIMATION OF METFORMINHYDROCHLORIDE, SAXAGLIPTIN AND
DAPAGLIFLOZIN IN THEIR BULK AND PHARMACEUTICAL DOSAGE FORMS*

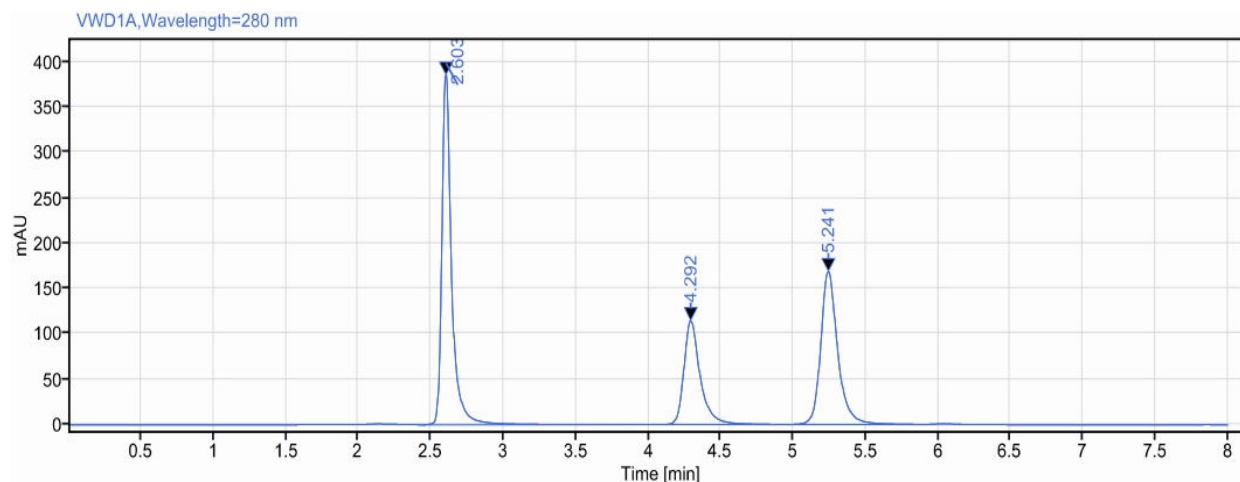*Section A-Research paper*

Figure-8: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin (Preparation-4)

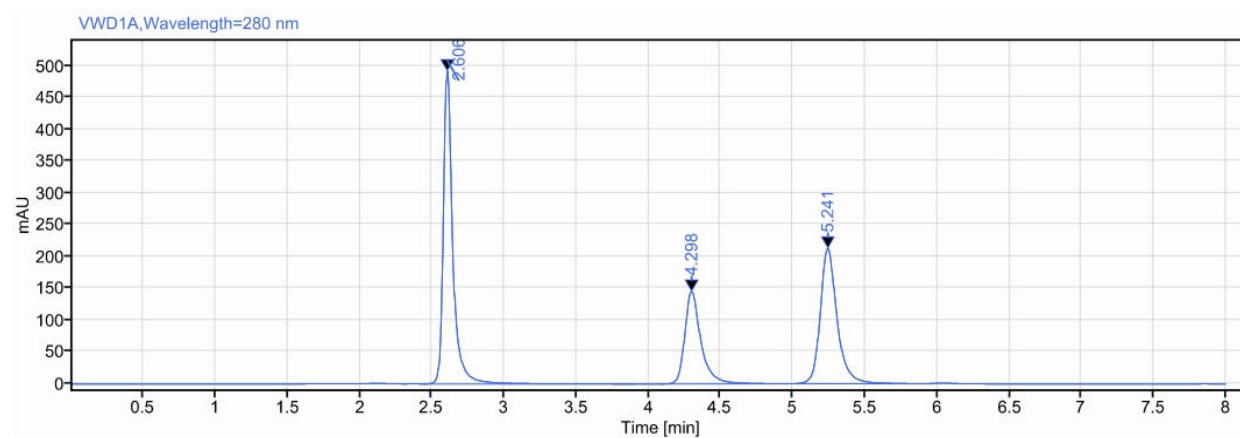Figure-9: Chromatogram of Metformin HCl, Saxagliptin and Dapagliflozin (Preparation-5)
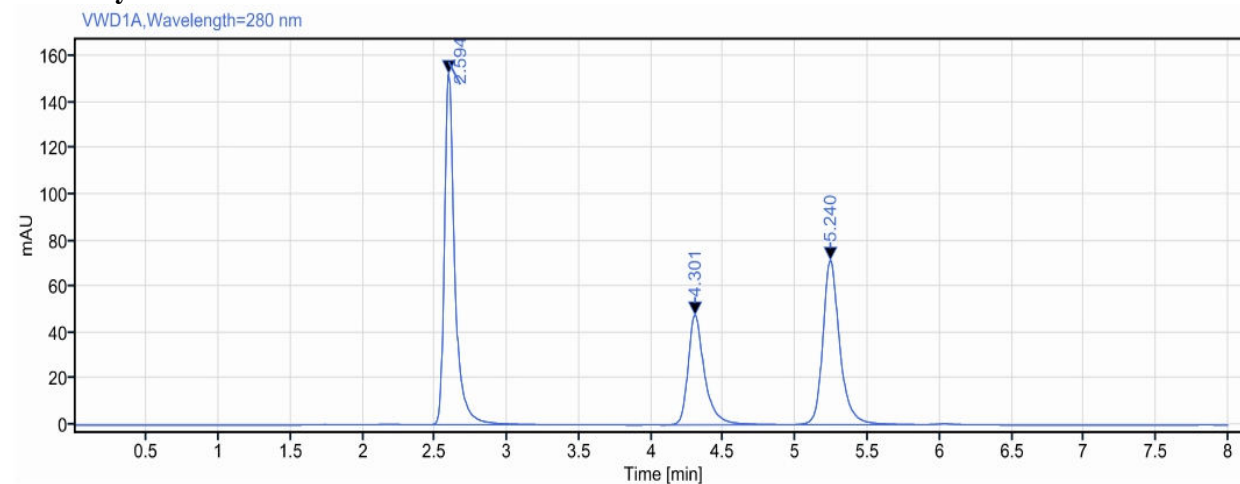
Accuracy:

Figure-10: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (50%, Preparation-1)

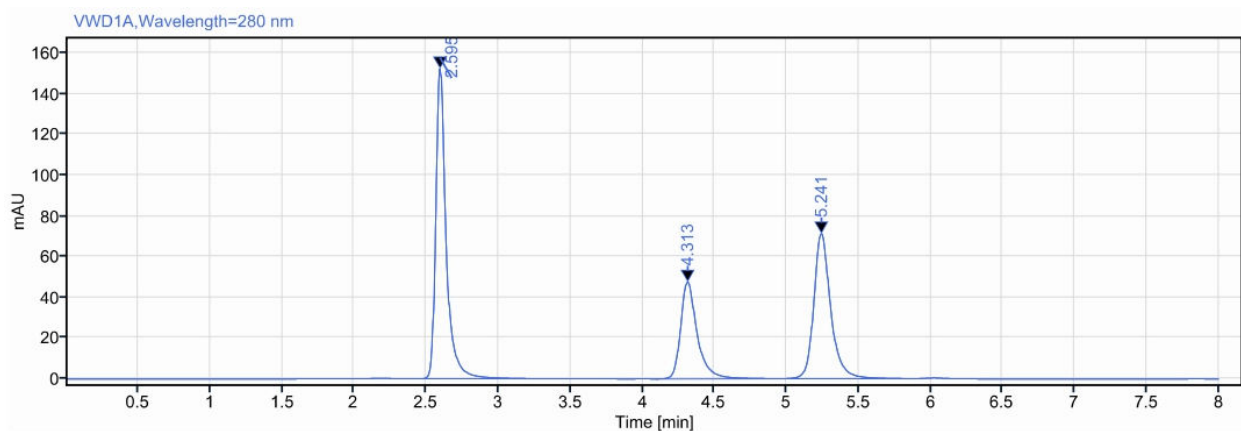| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.594		766.43	152.43	44.29	Metformin hcl	30176.14	1.50
4.301	10.82	381.29	47.58	22.03	Saxagliptin	30858.52	1.44
5.240	4.72	582.84	71.31	33.68	Dapagliflozin	42823.76	1.25
	Sum	1730.56					

Figure-11: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (50%, Preparation-2)

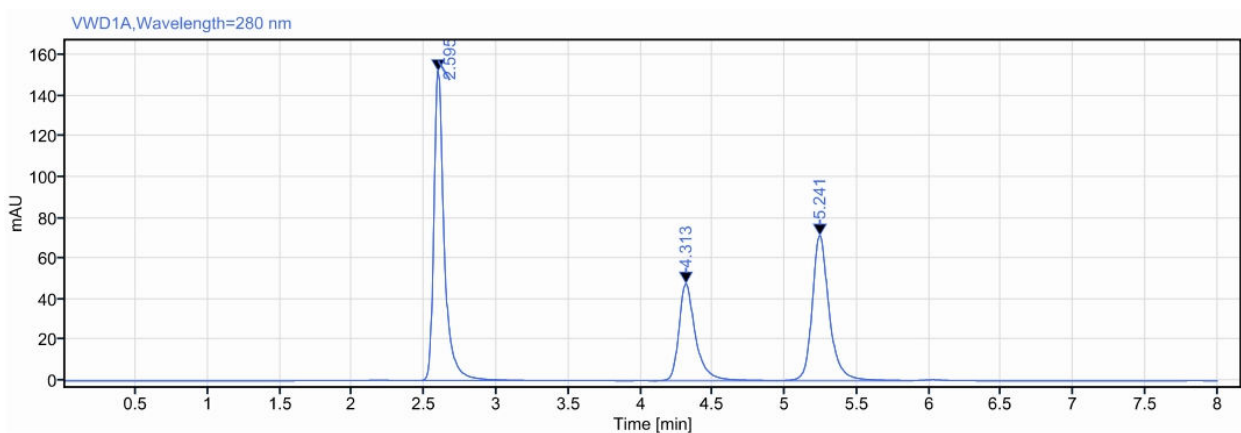| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.595		767.29	152.50	44.34	Metformin hcl	30152.81	1.49
4.313	10.89	380.99	47.63	22.02	Saxagliptin	31081.67	1.43
5.241	4.67	582.29	71.29	33.65	Dapagliflozin	42908.51	1.24
	Sum	1730.57					

Figure-12: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (50%, Preparation-3)

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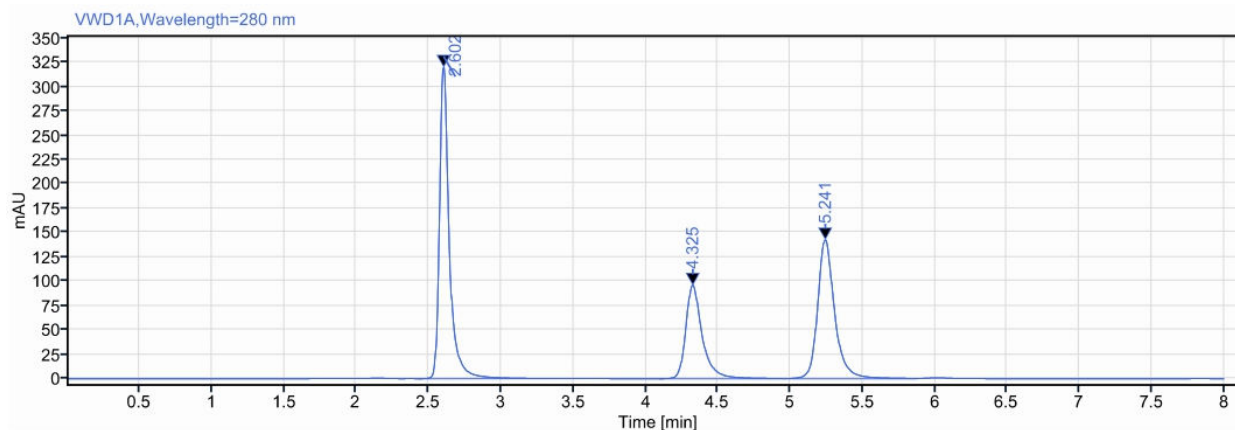| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.595		767.29	152.09	44.07	Metformin hcl	30152.81	1.49
4.313	10.89	384.09	47.64	22.06	Saxagliptin	31042.18	1.43
5.241	4.67	589.70	71.36	33.87	Dapagliflozin	42778.51	1.24
	Sum	1741.08					

Figure-13: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (100%, Preparation-1)

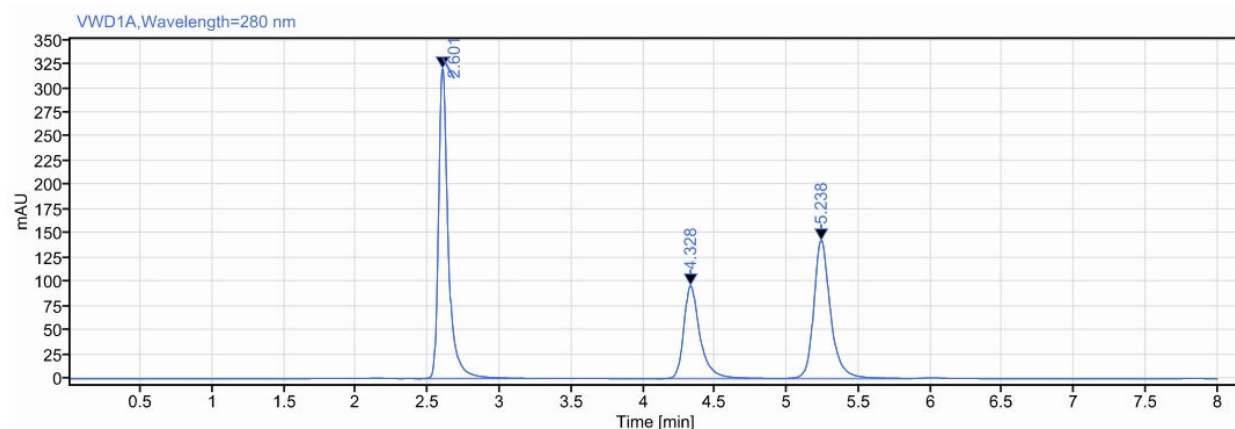| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.602		1516.69	320.36	43.94	Metformin hcl	35111.28	1.50
4.325	11.34	762.86	96.12	22.10	Saxagliptin	32205.86	1.40
5.241	4.63	1171.82	142.78	33.95	Dapagliflozin	43213.58	1.28
	Sum	3451.37					

Figure-14: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (100%, Preparation-2)

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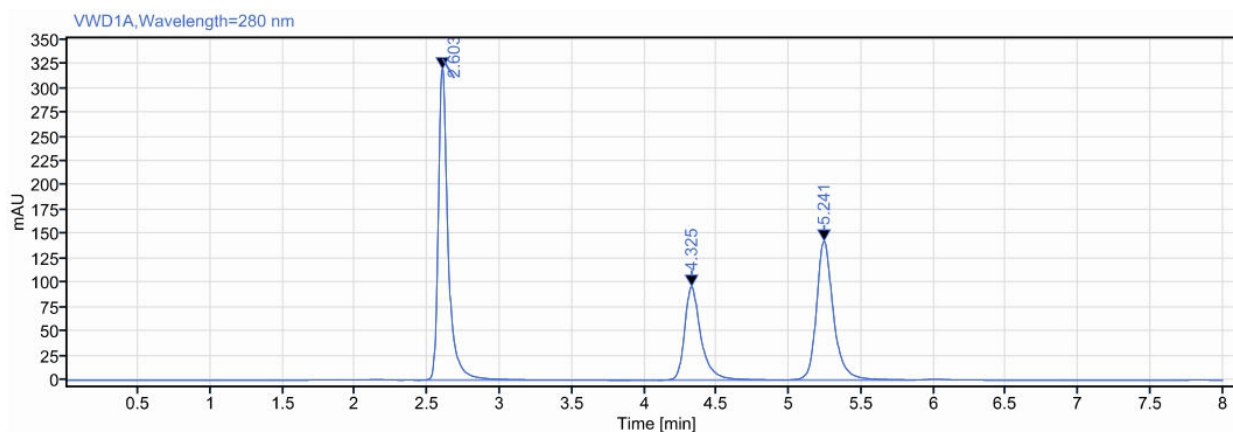| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.601		1514.56	320.49	43.89	Metformin hcl	35068.53	1.47
4.328	11.36	763.60	96.15	22.13	Saxagliptin	32233.71	1.41
5.238	4.61	1172.30	142.87	33.98	Dapagliflozin	43249.19	1.25
	Sum	3450.46					

Figure-15: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (100%, Preparation-3)

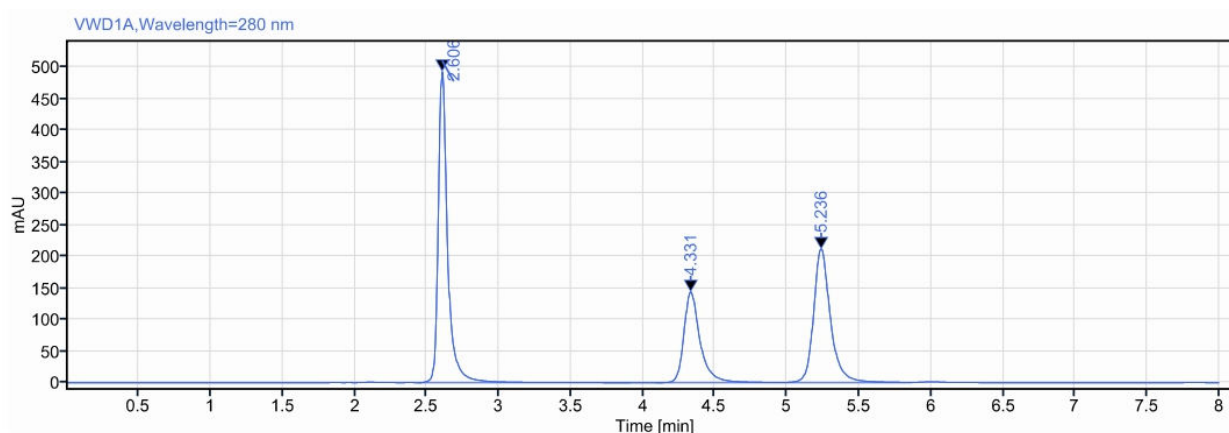| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.603		1519.61	319.47	43.99	Metformin hcl	35109.43	1.50
4.325	11.34	762.95	96.06	22.08	Saxagliptin	32205.19	1.40
5.241	4.63	1172.15	142.66	33.93	Dapagliflozin	43210.84	1.28
	Sum	3454.71					

Figure-16: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (150%, Preparation-1)

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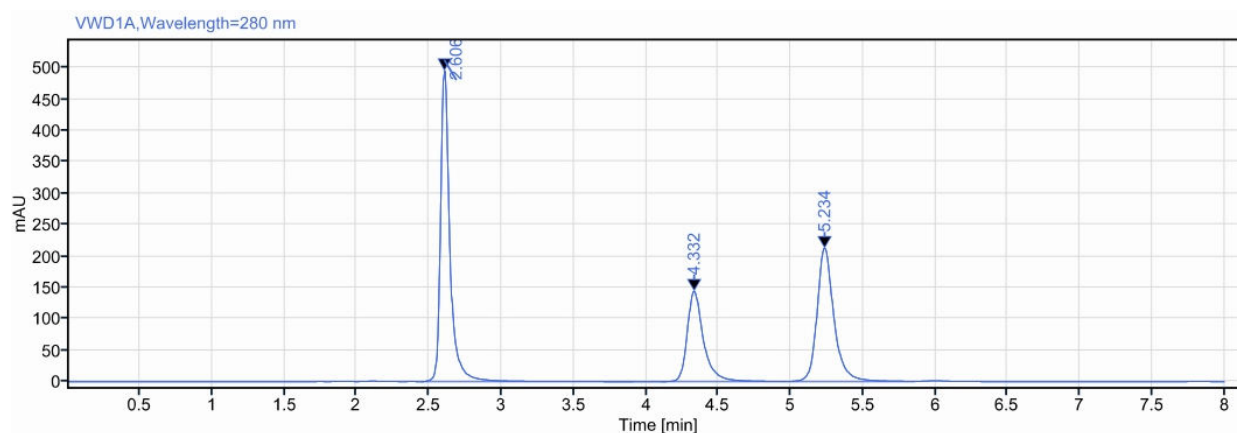| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.606		2253.80	494.42	43.95	Metformin hcl	38581.52	1.44
4.331	11.60	1134.01	144.18	22.11	Saxagliptin	32947.75	1.41
5.236	4.61	1740.23	212.36	33.94	Dapagliflozin	43353.29	1.28
	Sum	5128.04					

Figure-17: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (150%, Preparation-2)

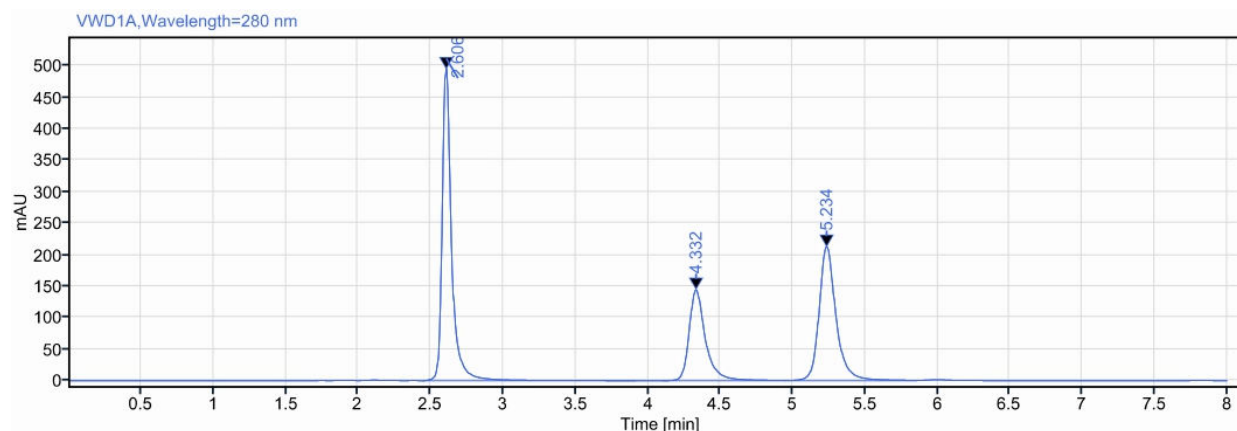| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.606		2253.42	495.68	43.95	Metformin hcl	38645.62	1.48
4.332	11.63	1134.21	144.39	22.12	Saxagliptin	33014.07	1.40
5.234	4.60	1740.02	212.60	33.93	Dapagliflozin	43398.10	1.27
	Sum	5127.66					

Figure-18: Chromatogram of accuracy of Metformin HCl, Saxagliptin and Dapagliflozin (150%, Preparation-3)

RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.606		2253.42	494.77	43.94	Metformin hcl	38645.62	1.48
4.332	11.63	1134.41	144.30	22.12	Saxagliptin	33013.09	1.40
5.234	4.60	1740.73	212.49	33.94	Dapagliflozin	43394.15	1.27
	Sum	5128.57					

Forced degradation studies:
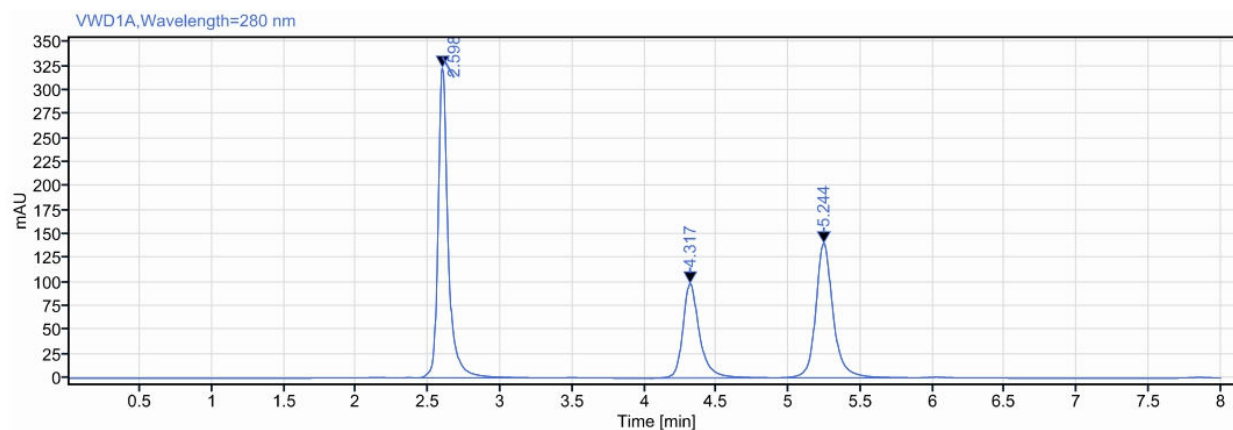## Acid degradation:

Figure-19: Chromatogram of Acid degradation

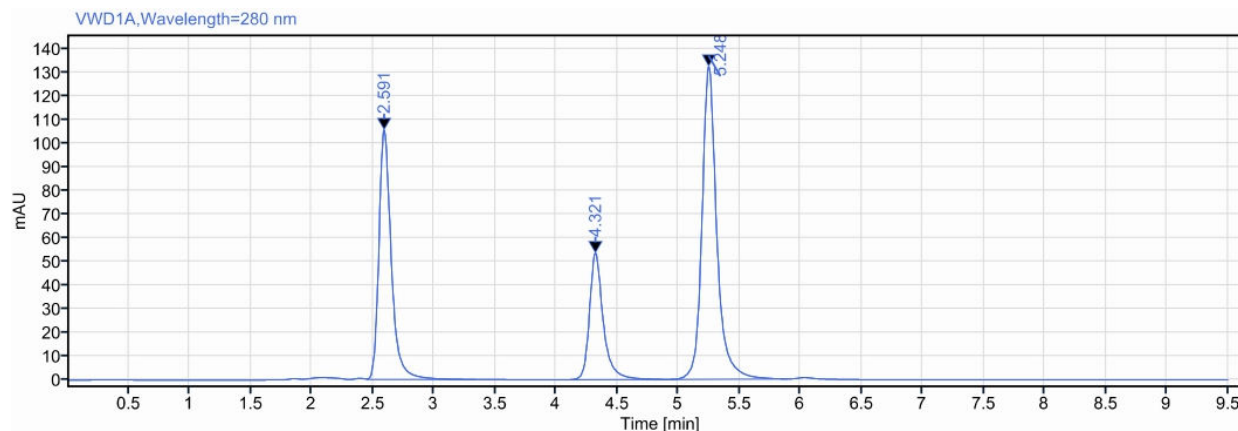| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.598		1582.70	323.54	44.53	Metformin hcl	33293.34	1.42
4.317	11.05	793.34	98.38	22.32	Saxagliptin	30604.26	1.33
5.244	4.59	1177.97	140.21	33.14	Dapagliflozin	41471.48	1.19
	Sum	3554.01					

Alkali degradation:

Figure-20: Chromatogram of Alkali degradation

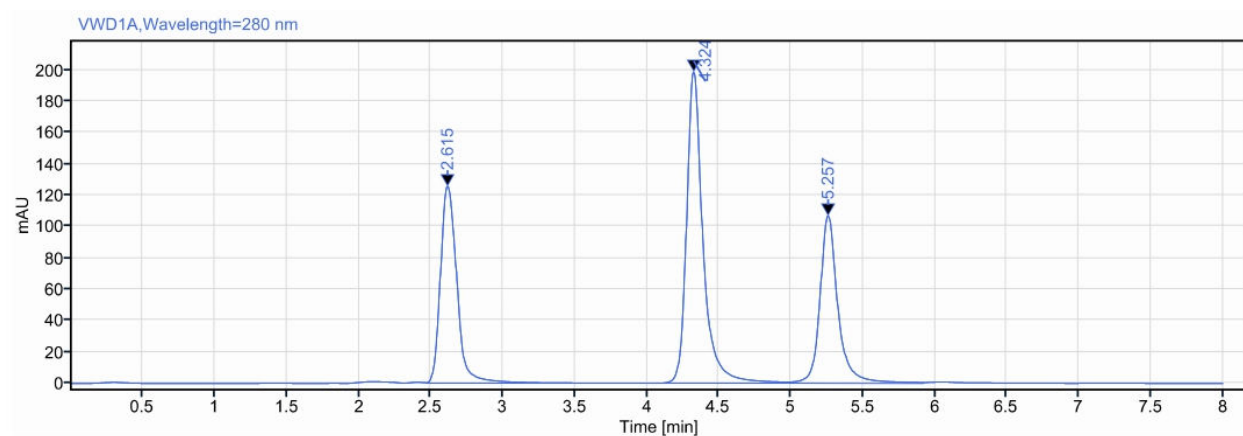| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.591		745.37	105.53	32.66	Metformin hcl	13748.43	1.38
4.321	9.39	422.34	53.53	18.50	Saxagliptin	32503.53	1.38
5.248	4.67	1114.72	132.20	48.84	Dapagliflozin	42014.76	1.25
	Sum	2282.43					

Peroxide degradation:

Figure-21: Chromatogram of Peroxide degradation

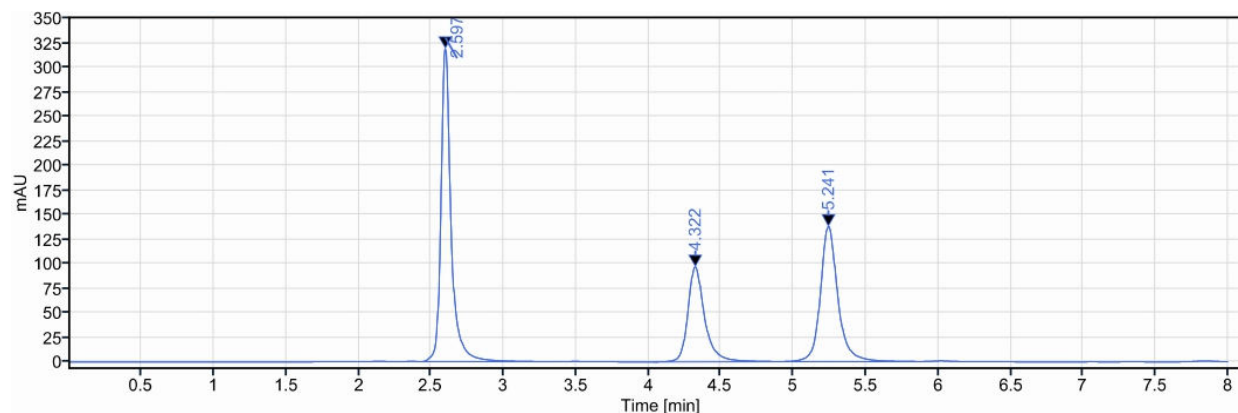| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.615		999.98	125.69	28.40	Metformin hcl	10666.32	1.26
4.324	8.68	1585.36	198.80	45.02	Saxagliptin	32998.54	1.40
5.257	4.69	936.27	107.00	26.59	Dapagliflozin	41168.36	1.25
	Sum	3521.61					

Photolytic degradation:

Figure-22: Chromatogram of Photolytic degradation

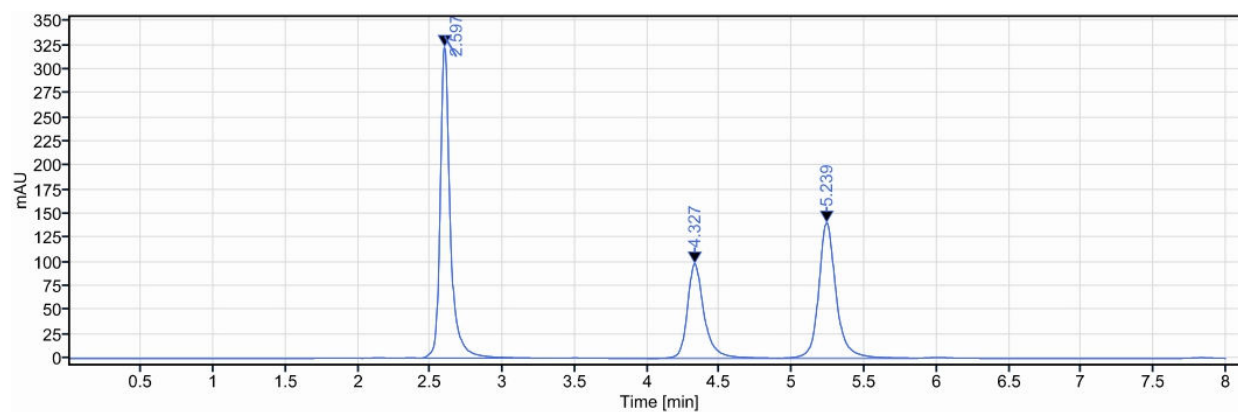| RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.597		1565.08	319.66	44.65	Metformin hcl	33400.07	1.38
4.322	11.08	781.59	96.89	22.30	Saxagliptin	30705.82	1.33
5.241	4.56	1158.74	137.91	33.06	Dapagliflozin	41515.94	1.18
	Sum	3505.41					

Thermal degradation:

Figure-23: Chromatogram of Thermal degradation

RT [min]	Peak Resolution USP	Area	Height	Area%	Compound Name	Peak Plates Per Meter USP	Peak Tail Factor
2.597		1578.32	322.77	44.23	Metformin hcl	33425.14	1.38
4.327	11.13	797.53	98.30	22.35	Saxagliptin	30841.24	1.31
5.239	4.52	1192.26	140.56	33.41	Dapagliflozin	41602.98	1.19
	Sum	3568.11					

CONCLUSION

As per the findings of the study for the chosen combination, sample recoveries in all formulations were in line with the claims made on each drug's label, and the suggested method was found to be accurate, sensitive, quick, and affordable for the detection of Metformin hydrochloride, Saxagliptin and Dapagliflozin in combined pharmaceutical formulations. Satisfactory findings were obtained after validating the suggested approach in accordance with guidelines of ICH and comparing the acquired values with the standard values. As a result, the technique can be used to determine the combination dosage form of Metformin hydrochloride, Saxagliptin and Dapagliflozin.

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