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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION AND SIMULTANIOUS ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN PHARMACEUTICAL DOSAGE FORM

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Abstract:

A straightforward, precise, exact technique was developed for the evaluation of the Dapagliflozin&Saxagliptinin tablet dosage form. Chromatogram was run through Altima 150 x 4.6 mm, 5µ. Mobile phasecarryingBuffer0.1%OPA:ACN in use in the %50:50was injecting into column at a flow rate of 1ml/min and 220nm. Runtime of Dapagliflozin&Saxagliptin was initiate to 2.691min and 2.143min. %RSD of the drugs were and get to be 0.8and 0.5 in the same way. %Recovery was get as 100.42% and 100.15%. LOD, LOQ values get from regression sum of Dapagliflozin&Saxagliptin were 0.52, 1.57 and 0.48, 1.44 likewise. Regression sum of Dapagliflozin is y = 11821x + 1957, and y =7209x + 2179 of Sitagliptin. The drugs were observed to be stable and less apt to degradation when they are subjected to a variety of stress conditions.

Key Words: *Dapagliflozin, Sitagliptin, RP-HPLC.*

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INTRODUCTION [1-15]:

CHROMATOGRAPHY

Itis a technique for the parting of a alloy by passing it in solution or suspension through a medium in which the analytes move at various rates.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

HPLC is Utilize ful for disconnecting particles or molecules that are separated in a dissolvable. Partition depends on adsorption, molecule exchange, partitioning of solid & liquid phase. Hplcconsume less run time, Regardless, stream rates were clashing, and the subject of whether it was more brilliant to have constant flow rate. HPLC instantly upgraded with the enhancement of segment squeezing materials. Additional convenience of on-line discoverers ended up being rapidly an incredible segment technique and is today called as HPLC.

Size exclusion chromatography:

This involves a solid stationary phase with controlled pole size. Solids are splitas per molecular size, with the big molecule unable to enter the surface release early.

ANALYTICAL METHOD DEVELOPMENT [17-21]:

Steps involved in method optimization Documentation starts at the simple start of the step upprocess. A structure for full documentation of upgrading considers must be set up. All data identifying with these investigations must be recorded in study facility scratch pad or an electronic database.

Sample preparation:

- ✓ Does the example require disintegration, filtration evacuation, preconcentration or tidy up,
- ✓ Is synthetic derivatization basic to help discovering affectability or selectivity?

ANALYTICAL METHOD VALIDATION [16-19]:

Apart ICH rules validation is "Establishing documented evidence, which impart a high level of word that a exact motion will always create a preferred result or product meeting its determined provision & quality itself".

System Suitability:

Daily test of molecules under HPLC technique and procedure are able of importing data of apt value.

Table 1 System Suitability Variables and their Limits

Variables	Limits
Capacity Factor (K)	K >2
Repeatability	RSD ≤ 2%
Relative Retention	Not vital as the resolution is confirmed
Resolution(R_S)	R_S of > 2
Tailing Factor(T)	T ≤ 2
Theoretical Plates(N)	> 2000

2. Linearity: It is a arithmetical connection that can be graphically constitute as a straight line.

3. Precision and accuracy: These are basic parts to their ability. Precision and accuracy submit to the robustness and reproducibility of an analytical process.

4. Specificity / selectivity: The word "selectivity" and "specificity" both provide the authenticity of the analytical process.

Selectivity is the capacity of the method to tell apart a specific analyte in a complex blend without interference from other analytes.

Specificity can be review as the ultimate selectivity, i.e. 100% selectivity (or 0% interferences).

6. Robustness: It can be defined as the skill to repeat the process in various labs or under various situations without the rate of unforeseen various in the founded result(s), trial system to assess the strength of a technique.

7. Limit of detection: LOD and LOQ are words used to explain the least concentration of molecule that can be consistently calculated by an analytical process.

The LOD calculated as:

$$LOD = 3.3\ \sigma / S$$

The LOQ calculated as:

$$LOQ = 10\ \sigma / S$$

σ = Standard deviation of Intercepts of calibration curves

S = Mean of slopes of the calibration curves

Characteristics	Acceptance Criteria
Accuracy/trueness	98-102%
Precision	%RSD < 2%
Repeatability	%RSD < 2%
Intermediate Precision	%RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	s/n > 2 or 3
Quantitation Limit	s/n > 10
Linearity	$R^2 > 0.999$
Range	80 –120 %

MATERIALS AND METHOD:

Material	Source
Reference sample(API)	Spectrum Pharmalabs, Hyderabad, Telangana
Test sample (QTERN Formulation)	Local pharmacy
HPLC grade :Acetonitrile, Methanol and water	Merck chemical division, Mumbai
AR grade: Potassium dihydrogen ortho phosphate, Ortho-phosphoric acid and sodium dihyrogen Ortho phosphate	Rankem, avantor performance material india limited

Instrument	Manufacturing company
Electronics Balance	Denver
Digital pH meter 7007	Digisun Electronics Hyderabad
Ultrasonicator	Labman
HPLC 2695 SYSTEM with PDA detector integrated with Empower 2 Software	WATERS
UV-VIS spectrophotometer integrated with UV win 6 Software	PG Instruments T60
Vacuum pump	Crompton
Hot Air Oven	Servewell Instrument PVT LTD, Bangalore.

RESULTS AND DISCUSSION:

Method development: technique development was finished by altering various, mobile phase ratios, buffers etc.

Trial 1:

Mobile stage	:	Water & Methanol (50:50)
stream rate	:	1 ml/min
Column	:	Agilent C18 (4.6 x 250mm, 5µm)
Detector wave length	:	220nm
Column temperature	:	30°C
Injection volume	:	10µL
Run time	:	10.0 min
Diluent	:	Water & Acetonitrile in the ratio 50:50
Results	:	Just Saxagliptin peak was eluted Dapagliflozin was not eluted so, further test is carried out.

Trial 2:

Chromatographic conditions:

Mobile phase	:	0.1% OPA: Acetonitrile (50:50)
stream rate	:	1 ml/min
Column	:	Azilent C18 (4.6 x 250mm, 5µm)
Detector wave length	:	220nm
Column temperature	:	30°C
Injection volume	:	10µL
Run time	:	10 min
Diluent	:	Water &Acetonitrile in the ratio 50:50
Results	:	Both peak were extraction but peak shape are bad Saxagliptin peak having tailing and lees plate count, Further test is carried out

Optimized method:

Chromatographic conditions:

Mobile phase	:	0.1% OPA buffer: Acetonitrile (50:50A)
stream rate	:	1 ml/min
Column	:	Altima C18 (4.6 x 150mm, 5µm)
Detector wave length	:	220nm
Column temperature	:	30°C
Injection volume	:	10µL
Run time	:	5min
Diluent	:	Water & Acetonitrile in the ratio 50:50
Results	:	Both peaks have good motion tailing factor, Usp plate count and motion

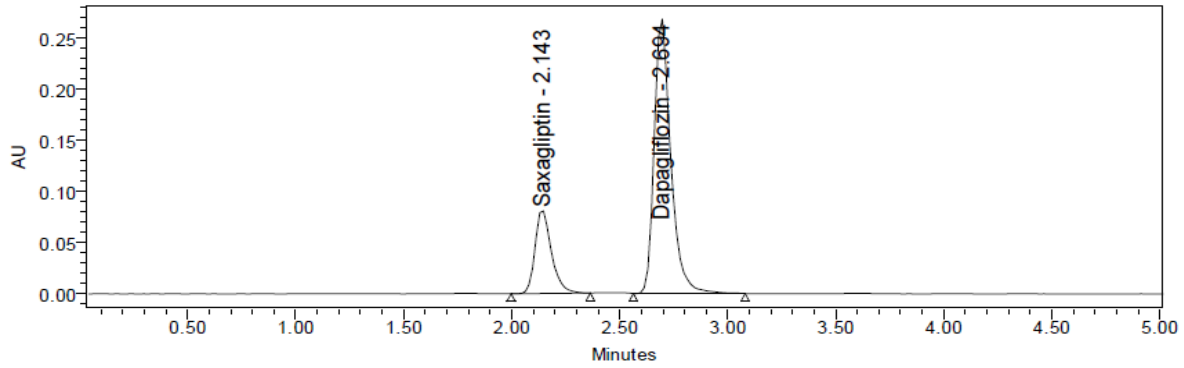

Fig 1:Optimized Chromatogram

Observation: Both peaks have excellent resolution. Plate count and tailing, so this technique was optimized and to be validated.

System suitability: Just as ICH all the variables were within limit.

Table:2 System suitability variables forDapagliflozin&Saxagliptin

S no	Saxagliptin			Dapagliflozin			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.139	3865	1.31	2.688	6160	1.33	3.9
2	2.140	3792	1.29	2.692	6503	1.32	3.9
3	2.143	4246	1.24	2.692	6699	1.31	3.9
4	2.144	4129	1.24	2.694	6553	1.27	3.9
5	2.144	3793	1.26	2.694	6522	1.31	3.9
6	2.144	4154	1.31	2.695	5810	1.36	3.8

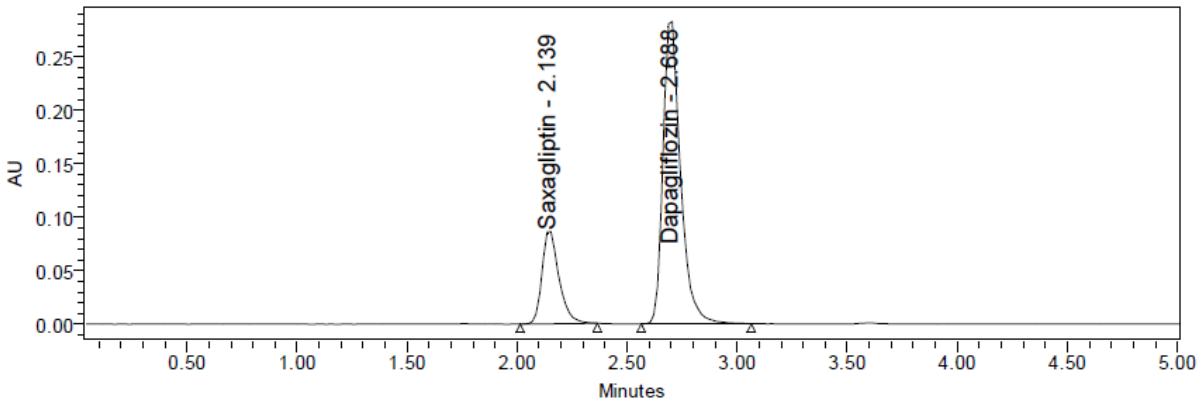

Fig 2: Systemsuitability Chromatogram

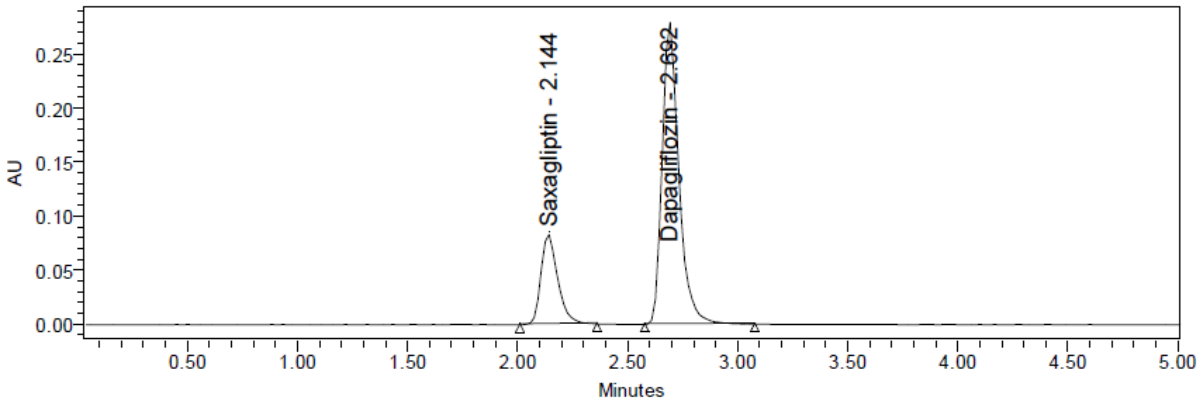

Fig 3: Systemsuitability Chromatogram

Discussion: Rt's were2.144min and 2.692 min. We did not get and prying peaks in blank and placebo at rt's of these drugs in this system.

Linearity:

Table 3: Linearity table.

DGFZ		SGPT	
Con (ppm)	**Peak area**	**Con (ppm)**	**Peak area**
25	294651	12.5	89265
50	590082	25	186149
75	899788	37.5	275460
100	1197550	50	368008
125	1456486	62.5	446397
150	1781199	75	542342

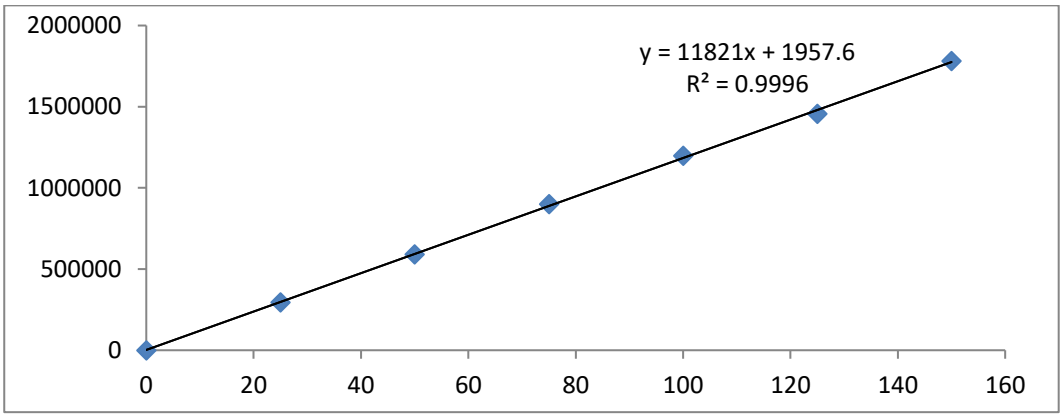

Fig 4: Calibration curve of DGFZ

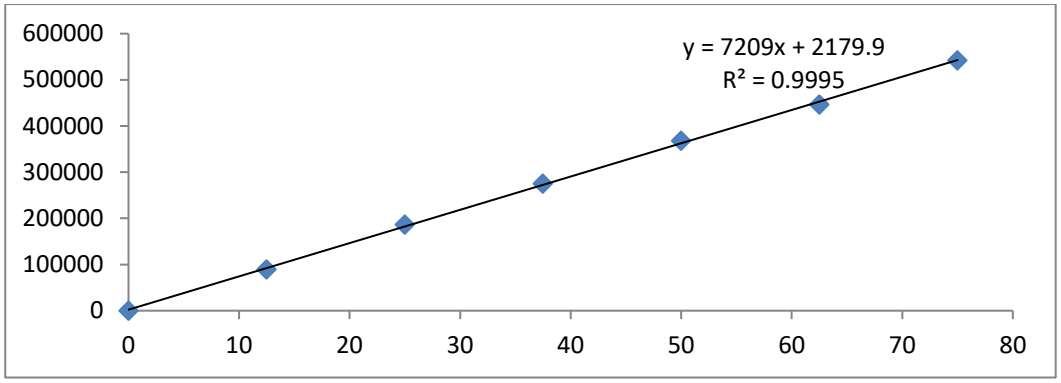

Fig 5: Calibration curve of SGPT

Discussion: Correlation coefficient get was 0.999 for the 2 drugs.

Precision:

System Precision:

Table 4: System precision table

S. No	Area of DGFZ	Area of SGPT
1.	1201654	368841
2.	1213258	370981
3.	1193339	367759
4.	1204565	370962
5.	1215082	367874
6.	1195609	370751
Mean	1203918	369528
S.D	8926.0	1549.2
%RSD	0.7	0.4

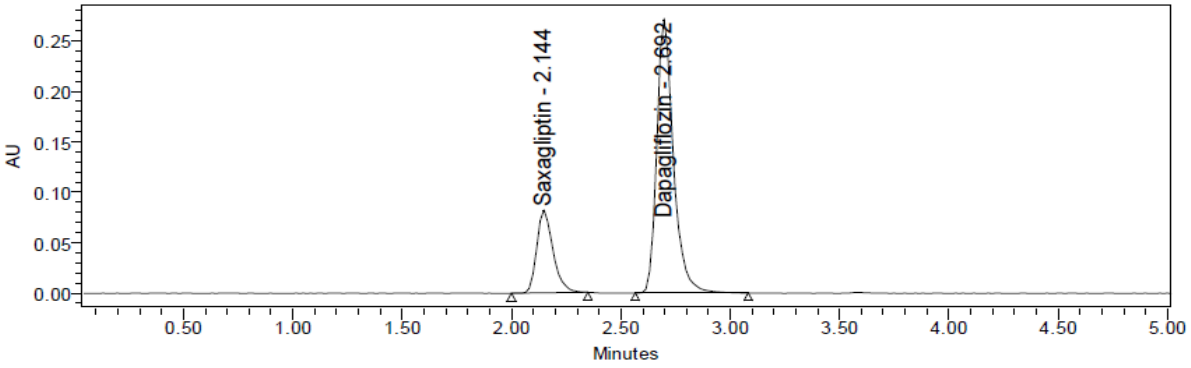

Fig 6: System precision chromatogram

Discussion: Mean area, SD and % RSD were calculated for 2 drugs.% RSDwere 0.7%and 0.4% likewise. These are within the limit.

Repeatability:

Table 5: Repeatability table

S. No	Area of DGFZ	Area of SGPT
1.	1210193	369417
2.	1187283	373212
3.	1191864	373152
4.	1208648	373803
5.	1203449	373811
6.	1206927	370690
Mean	1201394	372348
S.D	9537.5	1843.4
%RSD	0.8	0.5

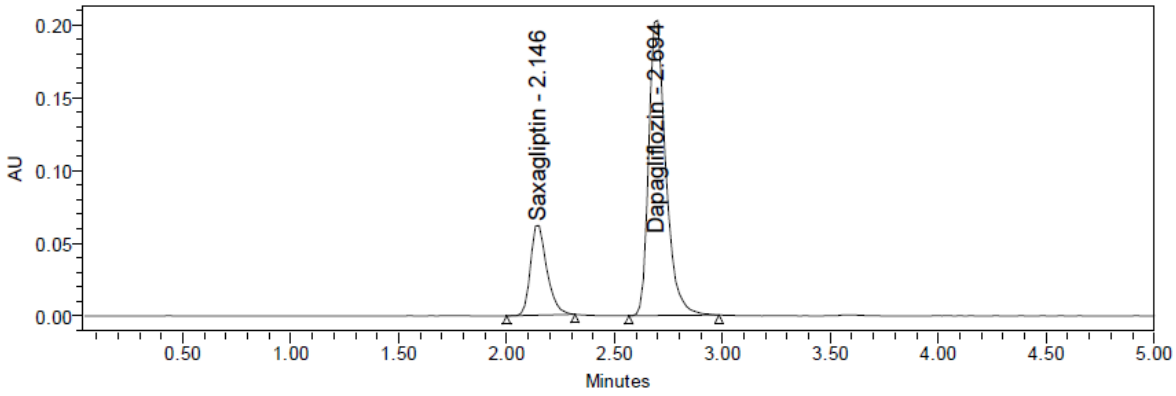

Fig 7: Repeatability chromatogram

Discussion: Mean area, SD&% RSD were calculated for 2 drugs and get as 0.8% and 0.5% likewise,within the limit

Day_Day Precision:

Table 6: Intermediate precision table

S. No	Area of DGFZ	Area ofSGPT
1.	1159517	348956
2.	1156853	344048
3.	1156956	345393
4.	1161823	343036
5.	1160918	342213
6.	1147114	348196
Mean	1157197	345307
S.D	5338.0	2756.2
%RSD	0.5	0.8

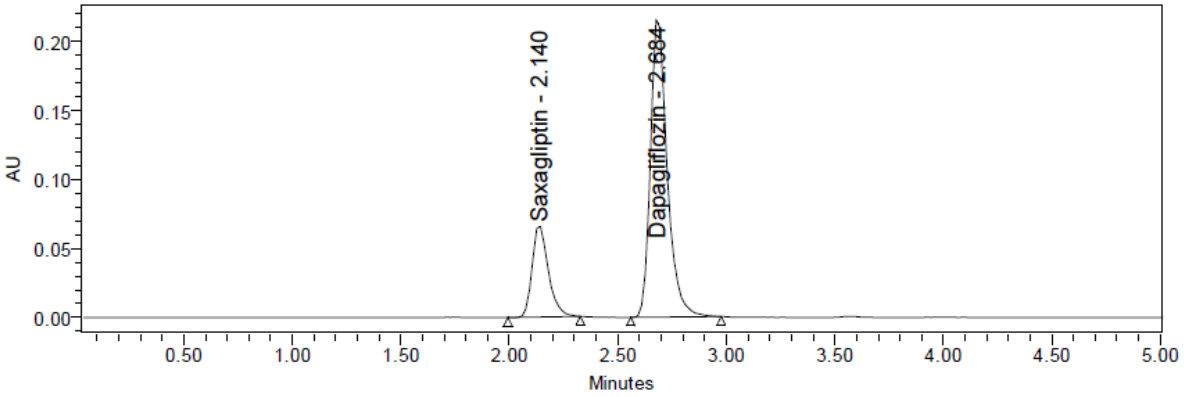

Fig 8:Inter Day precision Chromatogram

Discussion: Mean area, SD and % RSD were calculated for 2 drugs and get as 0.5% and 0.8%.

Accuracy:

Table 7: Accuracy table of Dapagliflozin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	50.261	100.52	100.42%
	50	50.460	100.92	
	50	50.201	100.40	
100%	100	100.486	100.49	
	100	101.242	101.24	
	100	100.170	100.17	
150%	150	150.120	100.08	
	150	149.608	99.74	
	150	150.318	100.21	

Table 8: Accuracy table of Saxagliptin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	25.23	100.91	100.15%
	25	24.85	99.42	
	25	25.00	100.02	
100%	50	49.58	99.17	
	50	50.74	101.47	
	50	49.84	99.67	
150%	75	75.23	100.30	
	75	74.44	99.26	
	75	75.84	101.12	

Discussion: 50%,100% and 150% solutions mean %Recovery was get as 100.42% and 100.15% .

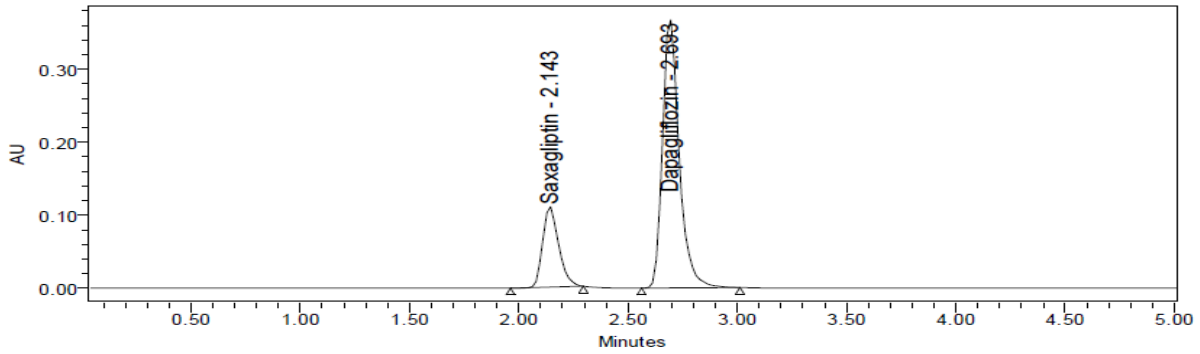

Fig 9: Chromatogramof Accuracy 50%

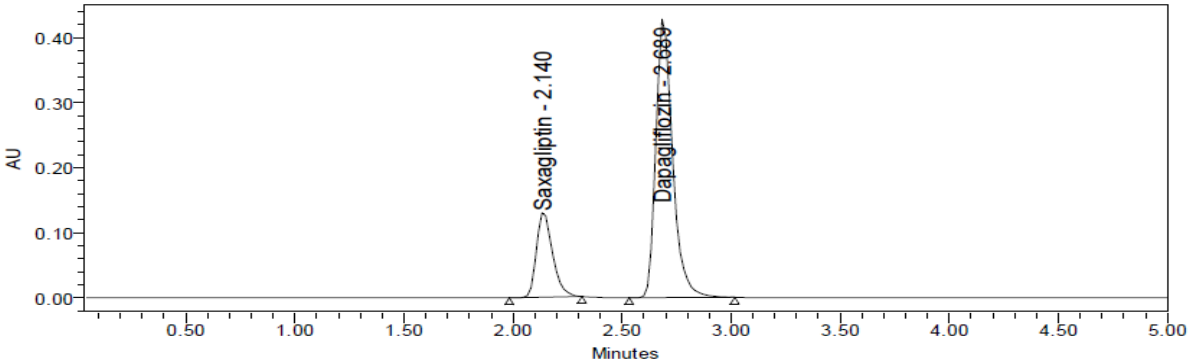

Fig 10:Chromatogramof Accuracy 100%

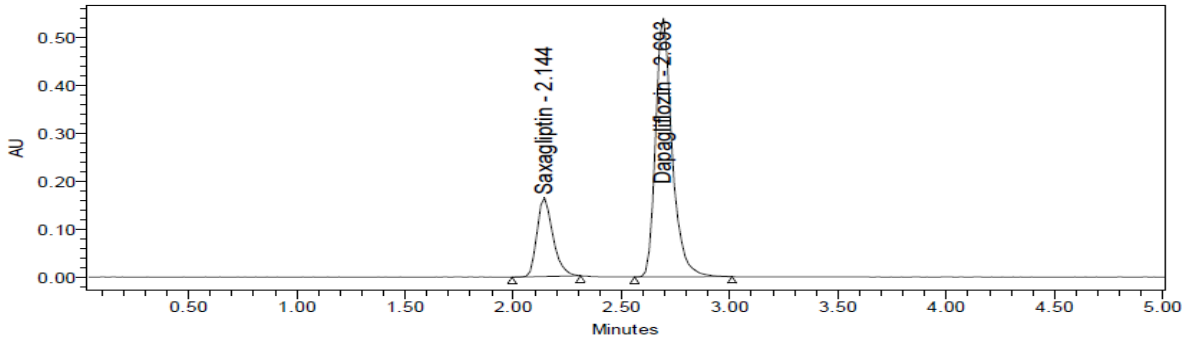

Fig 11: Chromatogram of Accuracy 150%

Sensitivity:

Table 9: Sensitivity table

Molecule	LOD	LOQ
Dapagliflozin	0.52	1.57
Saxagliptin	0.48	1.44

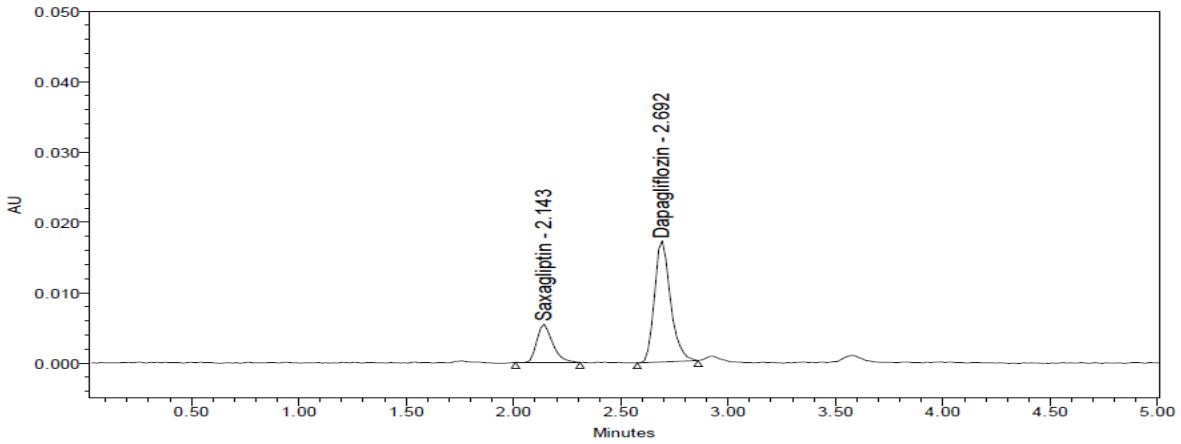

Fig 12:Chromatogram of LOD

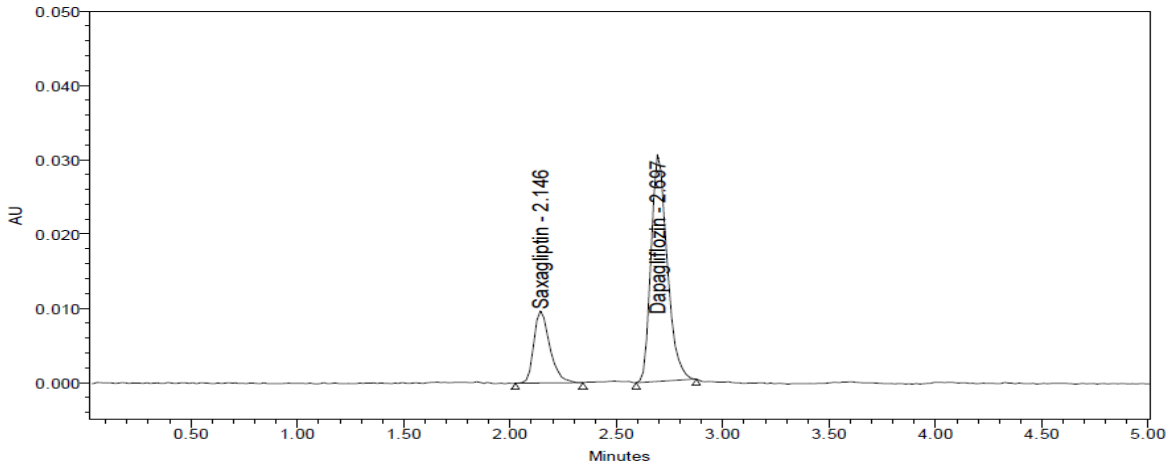

Fig 13: Chromatogram of LOQ

Robustness:

Table 10: Robustness data.

S.no	Condition	%RSD of Dapagliflozin	%RSD of Saxagliptin
1	Flow rate (-) 0.9ml/min	0.7	1.0
2	Flow rate (+) 1.1ml/min	0.3	1.5
3	Mobile phase (-) 60B:40A	0.4	0.8
4	Mobile phase (+) 50B:50A	1.2	0.8
5	Temperature (-) 25°C	0.4	0.4
6	Temperature (+) 35°C	0.5	1.5

Discussion: System aptnessvariables were not much affected and all the variables were approved. %RSD was within the limit.

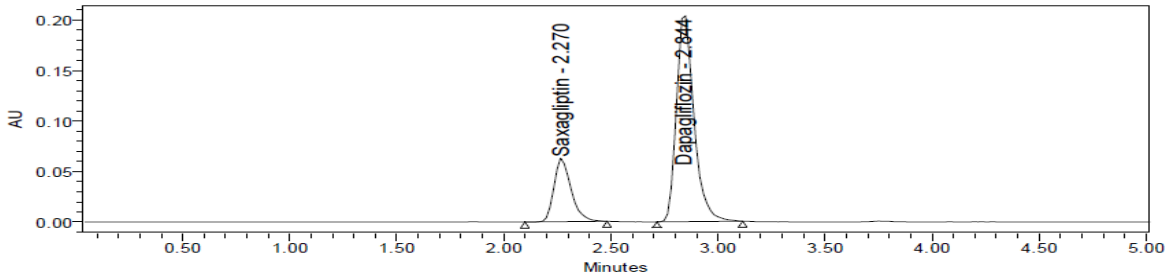

Fig 14: Flow minus

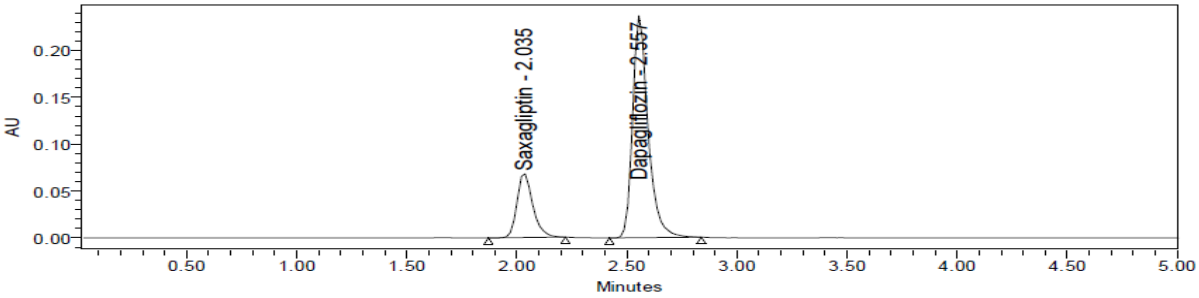

Fig 15: Flow plus.

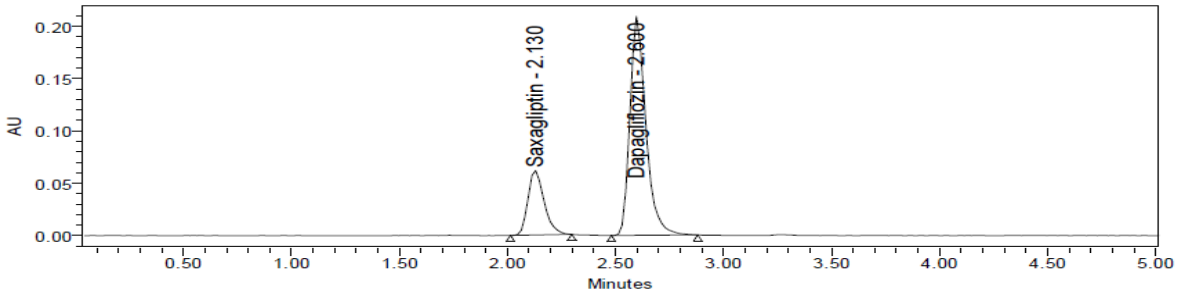

Fig 16: Mobile phase minus.

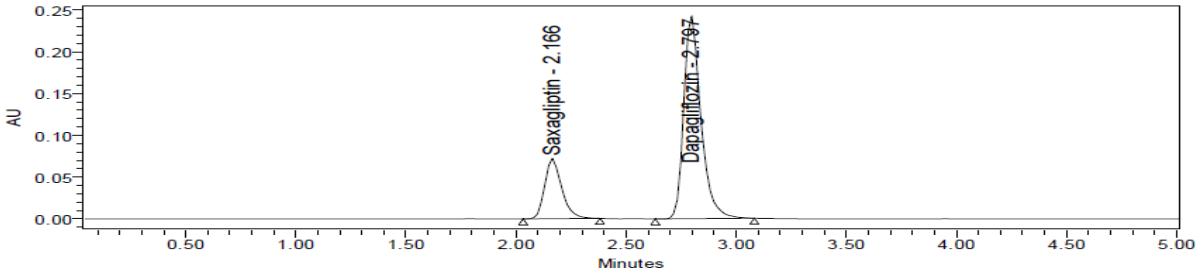

Fig 17: Mobile phase Plus.

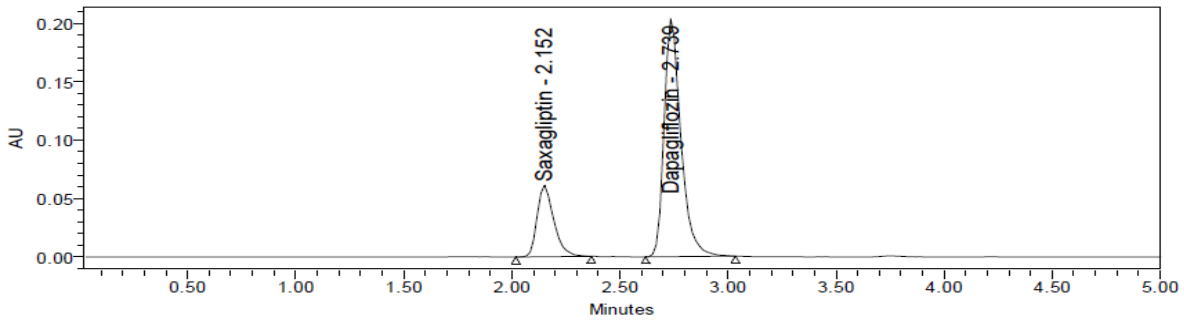

Fig 18: Temperature minus.

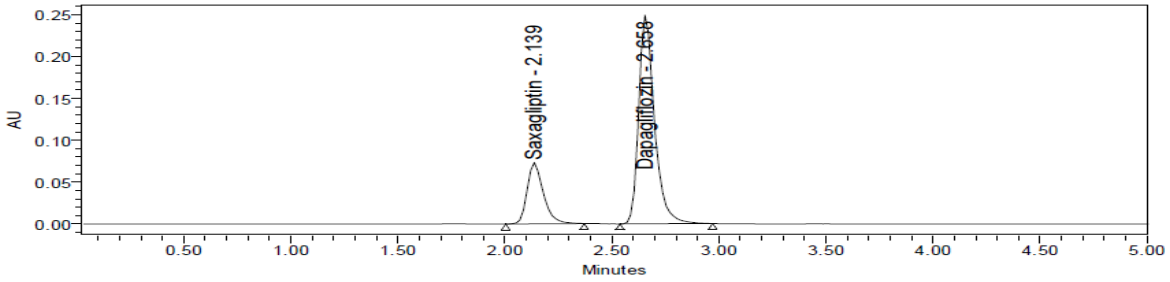

Fig 19: Temperature plus

Assay: AstraZeneca pharmaceuticals (Qtern), % Assay getwas 99.59% and100.36%likewise.

Table 11: Dapagliflozin of Assay Data

S.no	Standard Area	Sample area	% Assay
1	1201654	1210193	100.32
2	1213258	1187283	98.42
3	1193339	1191864	98.80
4	1204565	1208648	100.19
5	1215082	1203449	99.76
6	1195609	1206927	100.05
Avg	1203918	1201394	99.59
Stdev	8926.0	9537.5	0.8
%RSD	0.7	0.8	0.8

.**Table 12: Sitagliptin Assay Data**

S.no	Standard Area	Sample area	% Assay
1	368841	369417	99.57
2	370981	373212	100.59
3	367759	373152	100.58
4	370962	373803	100.75
5	367874	373811	100.75
6	370751	370690	99.91
Avg	369528	372348	100.36
Stdev	1549.2	1843.4	0.50
%RSD	0.4	0.5	0.5

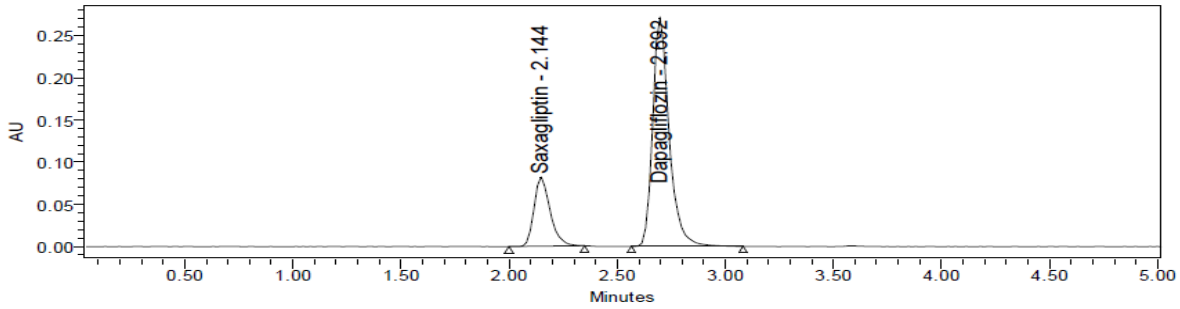

Fig 20: working standard solution

DEGRADATION

Table 13: Degradation Data of DGFZ

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.34	0.142	0.277
2	Alkali	4.09	0.145	0.276
3	Oxidation	3.82	0.130	0.286
4	Thermal	2.85	0.156	0.286
5	UV	1.60	0.156	0.280
6	Water	0.39	0.120	0.277

Table 14: Degradation Data of SGPT

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.89	0.215	0.469
2	Alkali	3.39	0.369	0.492
3	Oxidation	2.73	0.236	0.329
4	Thermal	2.42	0.441	0.499
5	UV	1.97	0.503	0.538
6	Water	1.97	0.533	0.600

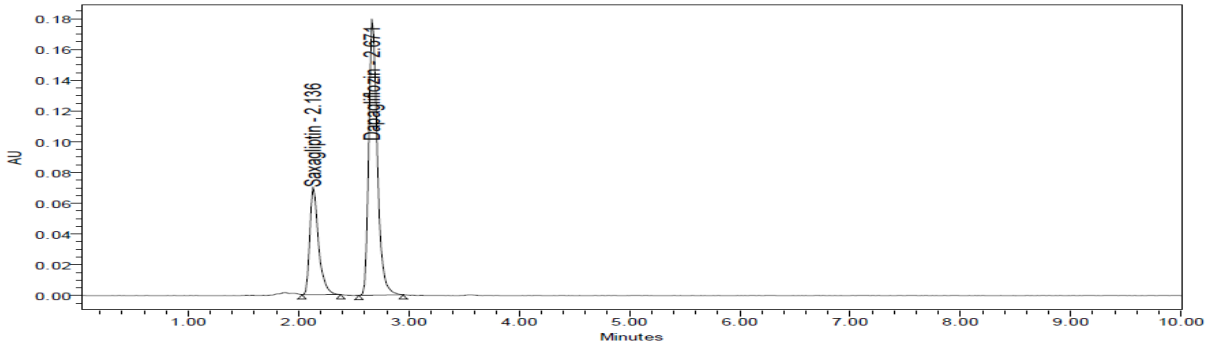

Fig 21: Acid chromatogram

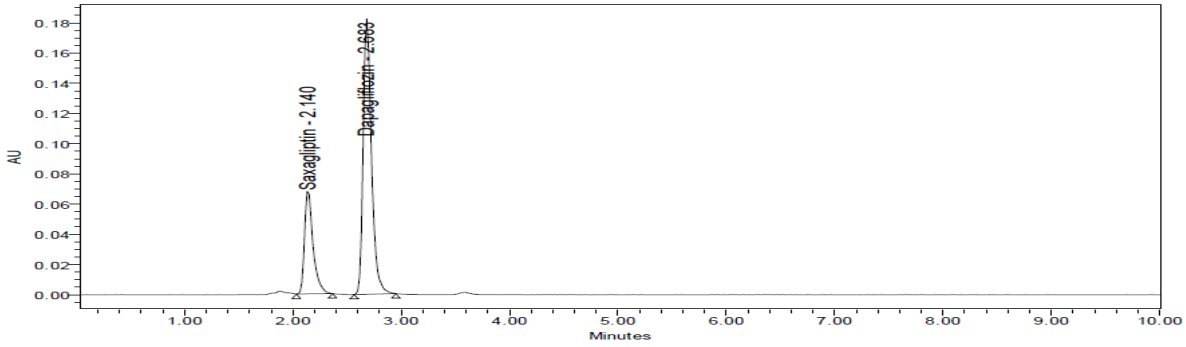

Fig 22: Base chromatogram

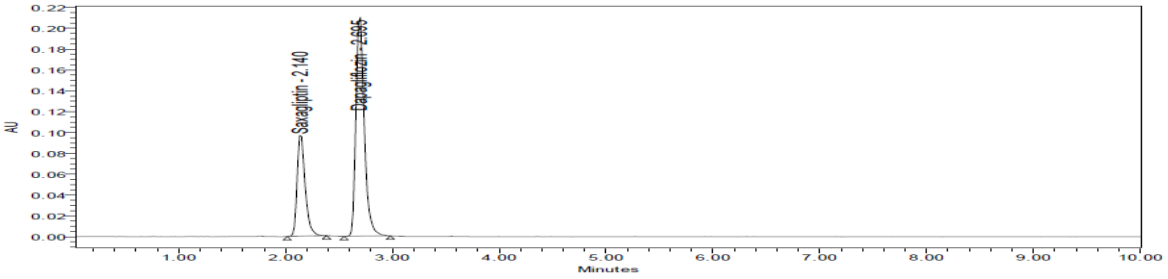

Fig 23: Peroxide chromatogram

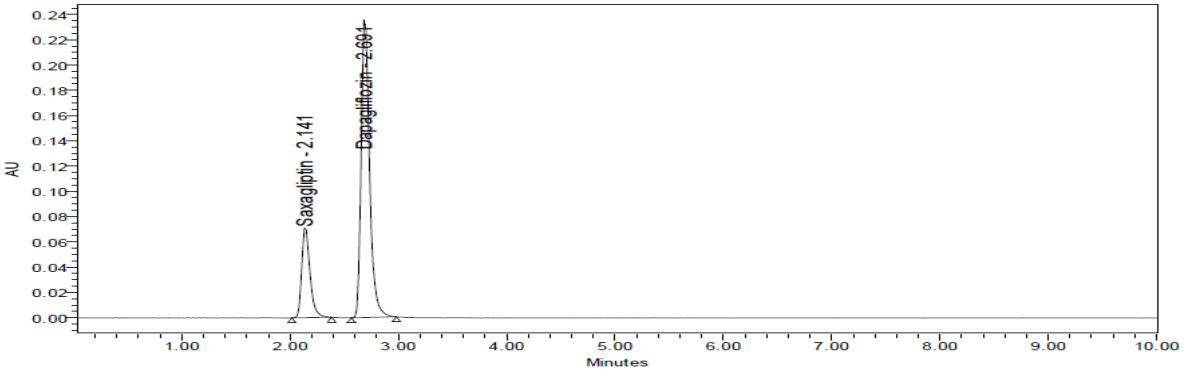

Fig 24: Thermal chromatogram

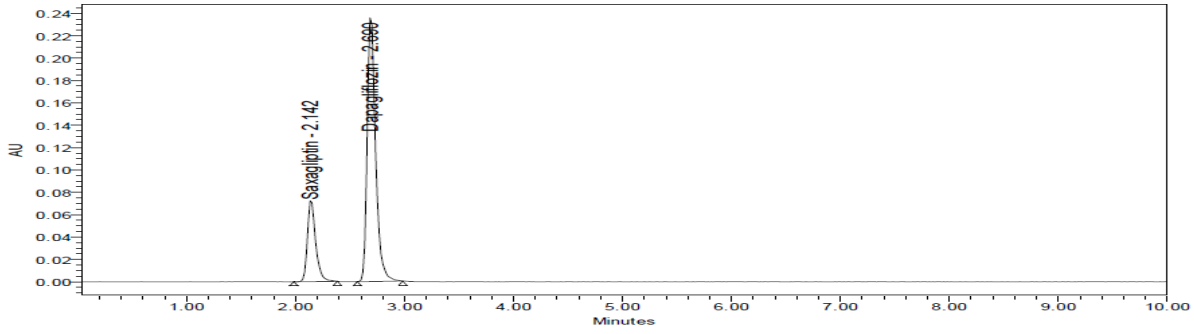

Fig 25: UV chromatogram

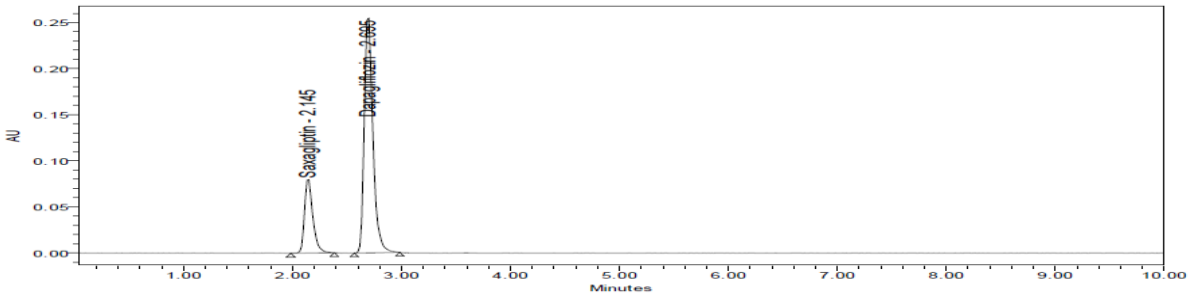

Fig 26: Water chromatogram

Discussion: All stress condition will not affect the technique

SUMMARY AND CONCLUSION:

Summary Table

Parameters		**Dapagliflozin**	**Saxagliptin**	**LIMIT**
Linearity Range(µg/ml)		25-150µg/ml	12.5-75 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		11821	7209	
Intercept(c)		1957	2179	
Regression equation (Y=mx+c)		y = 11821x + 1957	y =7209x + 2179	
Assay (% mean assay)		99.59%	100.36%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.7	0.4	NMT 2.0%
Method precision %RSD		0.8	0.5	NMT 2.0%
Accuracy %recovery		100.42%	100.15%	98-102%
LOD		0.52	0.48	NMT 3
LOQ		1.57	1.44	NMT 10
Robustness	**FM**	0.7	1.0	%RSD NMT 2.0
	FP	0.3	1.5	
	MM	0.4	0.8	
	MP	1.2	0.8	
	TM	0.4	0.4	
	TP	0.5	1.5	

CONCLUSION:

The developed technique is easy and has excellent accuracy and reproducibility. It can be used for the estimation of Dapagliflozin&Saxagliptin in bulk drug and in a dosage form. The technique was validated for linearity, accuracy, precision, ruggedness, robustness LOD, LOQ and recovery. This techniquegivessuitable values of recovery.

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