



## II. MATERIALS AND METHODS

Methods: Instruments-Instruments:

HPLC –Waters Model NO.2690/5 series Compact System Consisting of

Inertsil-C18 ODS column.

Electronic balance (SARTORIOUS)

Sonicator( FAST CLEAN)

Substances containing chemicals:

Methanol HPLC Grade.

Raw equipment(Unprocessed materials):

Semaglutide is working standard.

## III. RESULTS AND DISCUSSION:

Preparation of Standard stock solutions:

Accurately Weighed and transferred 10mg of Semaglutidedrug in 10 ml Volumetric flask and add 7ml of Methanol and sonicate for 30 minutes. After 30 minutes add 3 ml of Methanol , make up to the mark and sonicate for 5 to 10 minutes.

Preparation of Standard working solutions (100% solution): Take 1 ml of above 1000ppm stock solution in 10 ml of volumetric flask, make up to the mark with Methanol and sonicate .

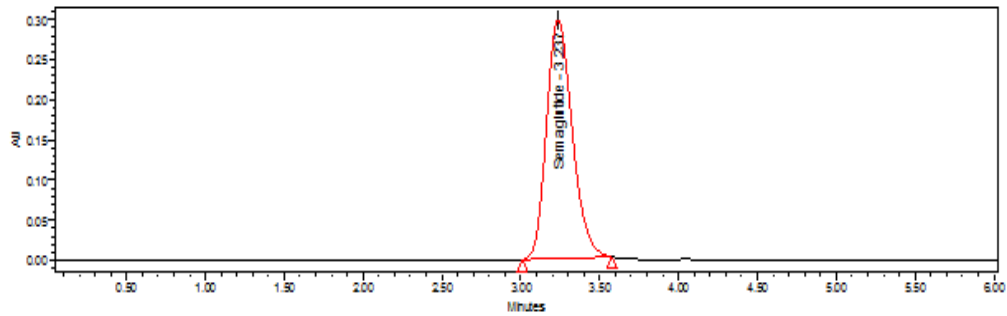
### 3.1 ADVANCED METHOD (OPTIMIZED METHOD)

Mobile Phase: Methanol: Water (70:30)V/V.

Chromatographic conditions that have been optimized:

Parameters	Method
Stationary phase (column)	Inertsil -ODS C <sub>18</sub> (250 x 4.6 mm, 5 μ)
Mobile Phase	Methanol: Water (70:30)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	5 min
Column temperature (°C)	Room temperature
Volume of injection loop (μl)	10 micro litres
Detection wavelength (nm)	274nm
Drug RT (min)	3.237min

Fig 7.1.1 Standard chromatogram



Inference: Got chromatogram at a Rt of 3.237 for standard 7.1.1

S.NO	Name of the peak	Retention time(min)
1	Semaglutide	3.237

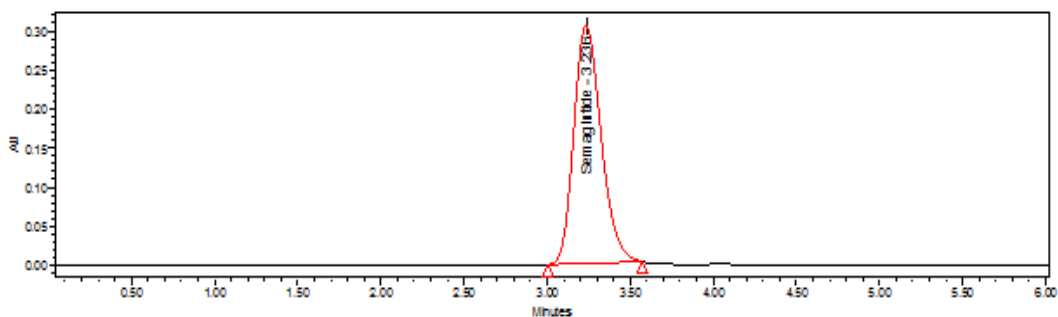
**7.2 INFORMATION OF HIGH VALUE (VALIDATION DATA)**

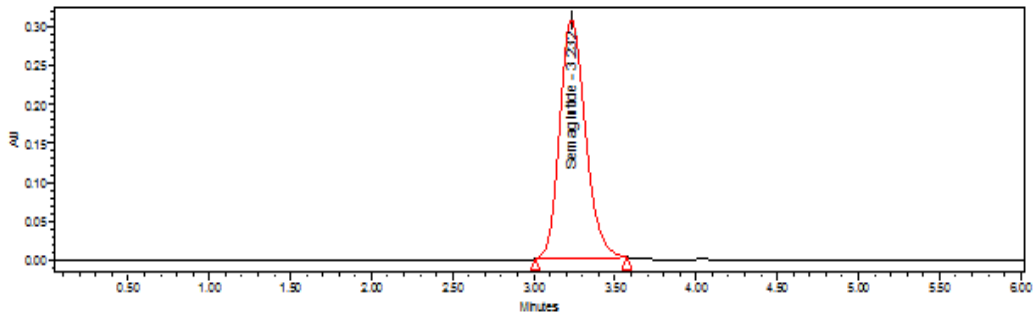
**7.2 PRODUCTS FOR THE SYSTEM (SYSTEM SUITABILITY):**

TABLE 7.2.1: Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.236	674753	10953.609752	1.153539
2	3.235	674261	10951.014286	1.155271
3	3.232	675298	10003.278630	1.157740
4	3.236	679221	10986.906427	1.159499
5	3.232	688636	10946.878423	1.152820
Mean	3.23422	678433.8	10768.34	1.155774
SD	0.00228	6031.135	-----	-----
% RSD	0.066247	0.888979	-----	-----

Fig: 7.2.1 System suitability chromatograms (standards)





Inference: Standard Chromatogram-1 System Suitability.

Inference: For standard Chromatogram-2, a suitable system is required.

DESCRIPTION(SPECIFICITY):

Fig 7.3.1 Blank Chromatograph

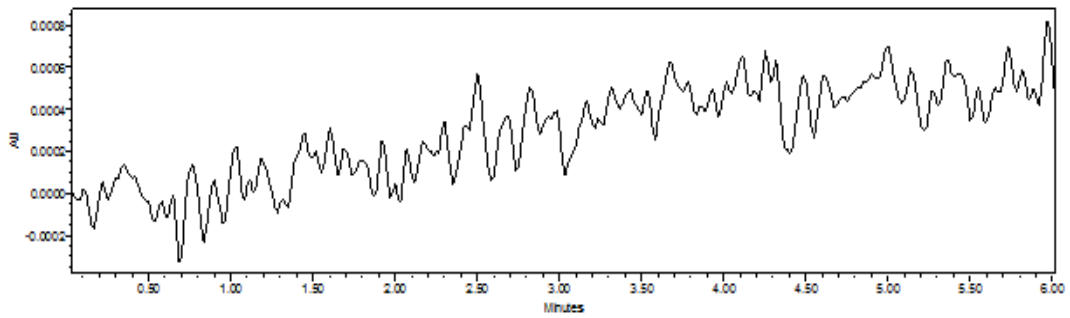
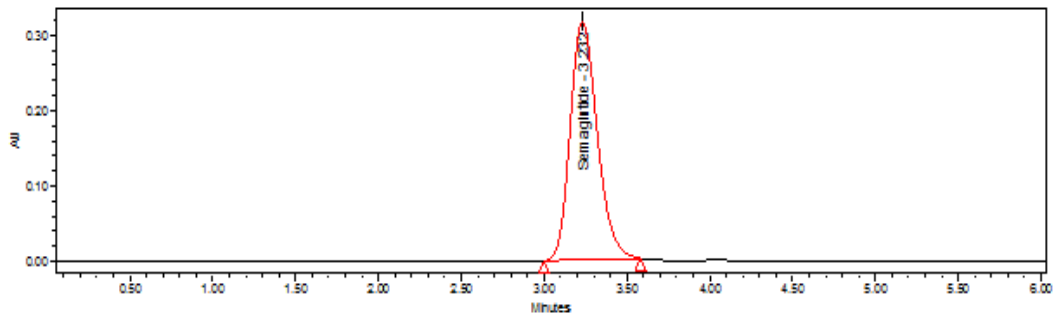


Fig 7.3.2: Chromatogram Standard



Inference: Got a peak for std at an Rt of 3.232min

7.4 PREQUIRE(PRECISION):

7.4.1 Repetition(Repeatability):

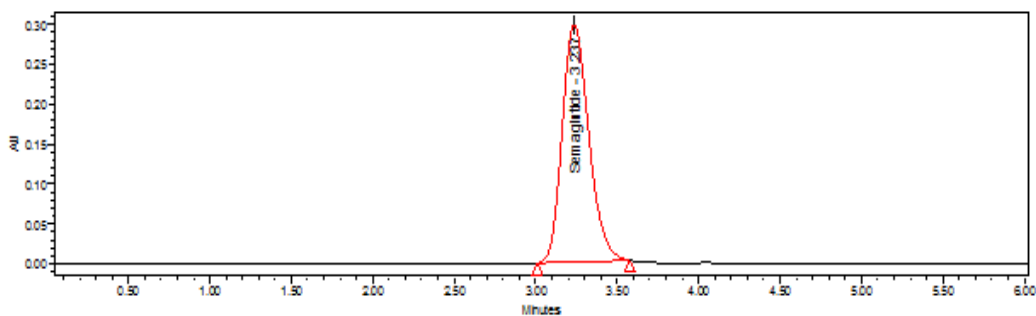
(a) Precise system(System precision):

TABLE-2: Data of Repeatability (System precision)

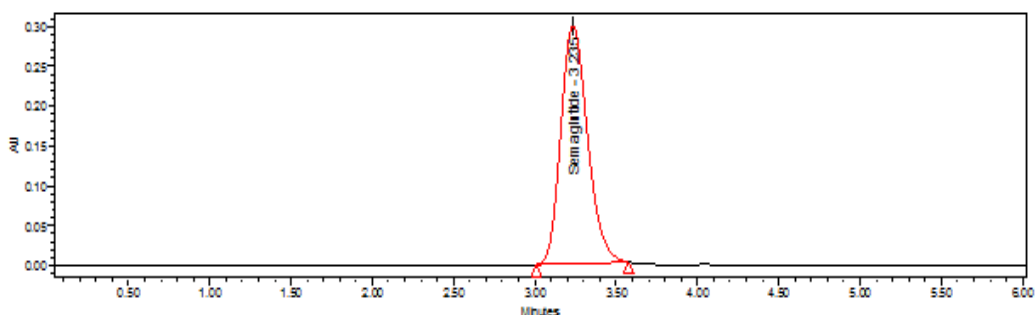
	Injection	Peak Areas of Semaglutide	% Assay
Concentration 40ppm	1	674753	98.66
	2	674261	99.30
	3	675298	101.53
	4	679221	100.53
	5	688636	99.98

Statistical Analysis	Mean	678433.8	100.00
	SD	6031.135	1.107678
	% RSD	0.888979	1.10

Fig 7.4.1 Detailed chromatograms of systems



Inference: Precision chromatograph devices (standard-1)

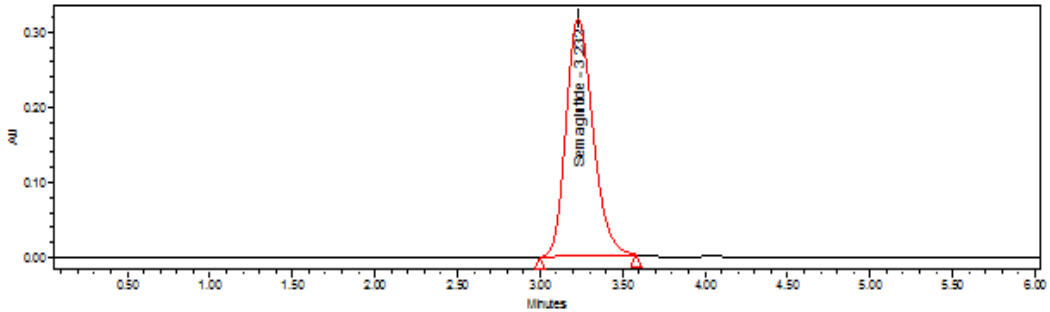


7.5 (b) Method precision:

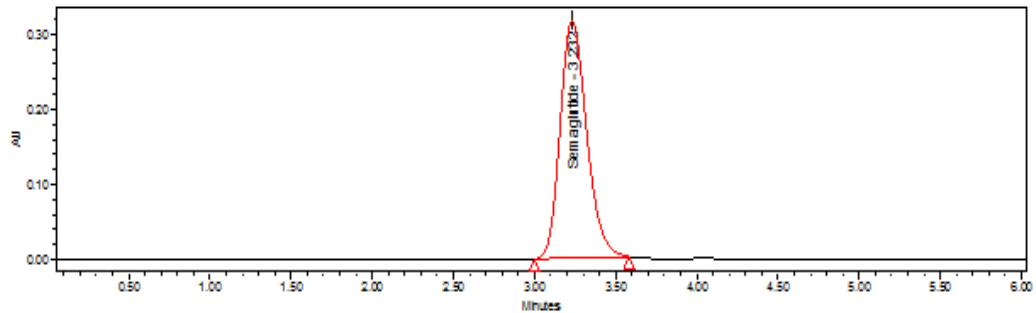
TABLE-7.5.1: Data of Repeatability (Method precision)

	Injection	Peak Areas of Semaglutide	% Assay
Concentration 40ppm	1	633495	98.55
	2	635992	98.88
	3	639828	99.40
	4	639098	99.30
	5	648289	100.53
	6	631322	98.28
Statistical Analysis	Mean	637312	99.278
	SD	5988.879	0.827236
	% RSD	0.0891	0.83

Fig 7.5.1: Repeatability chromatograms (Repeatable Chromatograms)



Inference: Chromatograph with high repeatability (Standard-1)

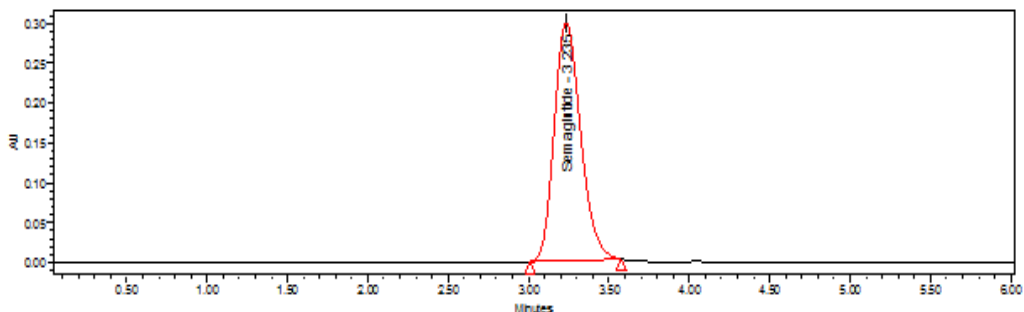


7.6 Intermediate precision:

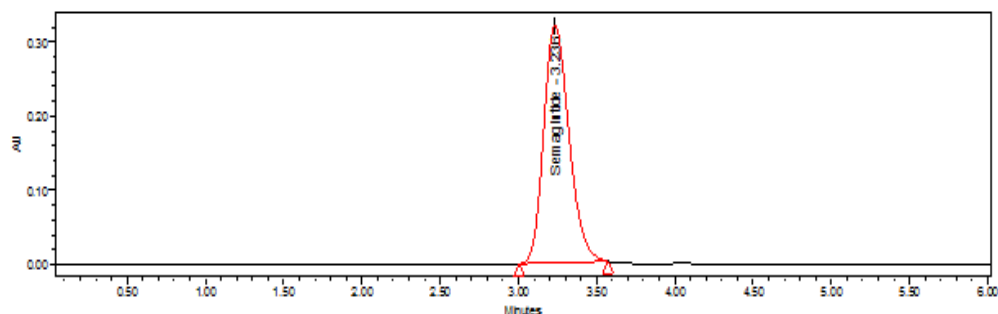
Table 7.6.1 Data of Intermediate precision (Analyst 2)

	Injection	Peak Areas of Semaglutide	% Assay
Concentration 40ppm	1	636792	99.99
	2	634360	99.66
	3	655696	101.53
	4	644147	99.98
	5	644127	99.97
	6	652525	101.10
Statistical Analysis	Mean	644607.8	100.37
	SD	6392.59	0.753536
	% RSD	1.183	0.75

Fig 7.6.2: Chromatograms of Intermediate Precision



Inference: Chromatograph with a medium precision.1

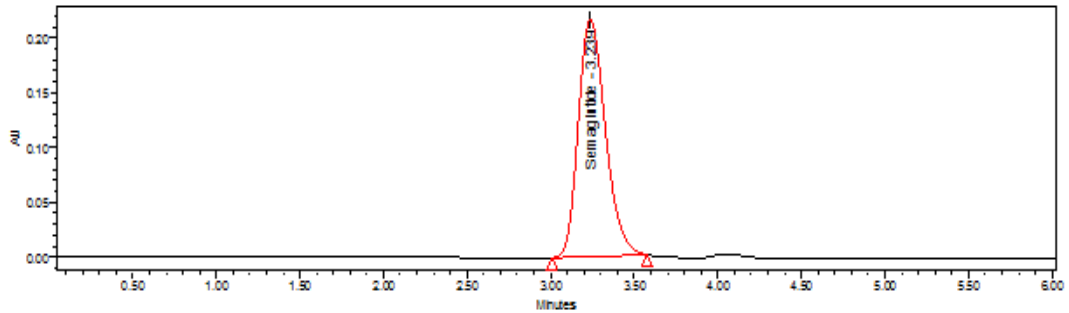


### 7.7 Resilience (ACCURACY)

TABLE 7.7.1: Data of Accuracy

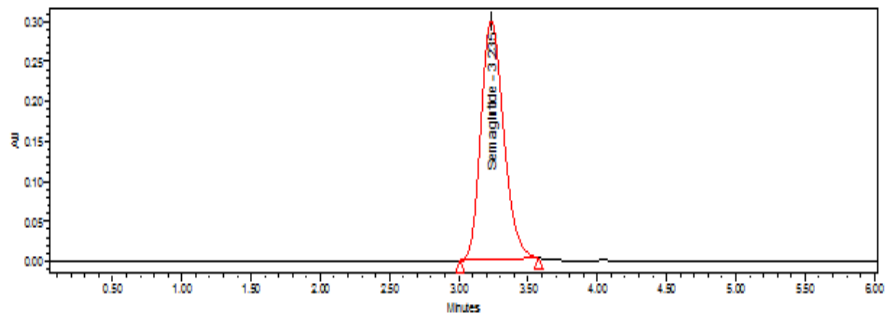
Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	20.04	100.22	MEAN	100.06
50% Injection 2	20	19.97	99.85	%RSD	0.18
50% Injection 3	20	20.02	100.11		
100 % Injection 1	40	40.01	100.02	MEAN	100.04
100 % Injection 2	40	40.05	100.14	%RSD	0.091
100% Injection 3	40	39.98	99.96		
150% Injection 1	60	60.08	100.14	MEAN	100.02
150% Injection 2	60	59.97	99.96	%RSD	0.09
150% Injection 3	60	59.98	99.98		

Fig 7.7.1 :Chromatographic precision (50 percent)

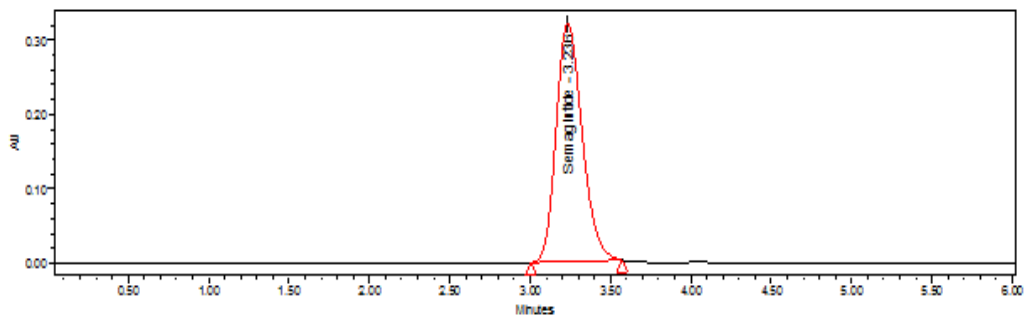


Inference: Standard 1 chromatogram

Fig 7.7.2: Chromatograms with extreme accuracy (100 per cent)



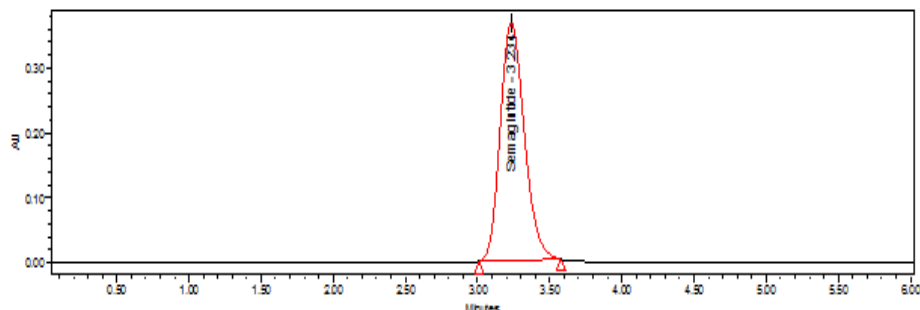
Inference: Standard 1 chromatogram



Inference: Standard 2 chromatogram



Fig 7.7.3: Chromatograms are used to ensure precision (150 per cent)



Inference: Standard 1 chromatogram  
 7.8 Variability (LINEARITY):

TABLE 7.8.1: Data of Linearity

Concentration (ppm)	Average Area	Statistical Analysis	
		0	0
20	632546	y-Intercept	276.2
30	658296	Correlation Coefficient	1
40	694400		
50	730308		
60	916282		
70	9402046		

7.8.2 Linearity plot (Concentration Vs Response)

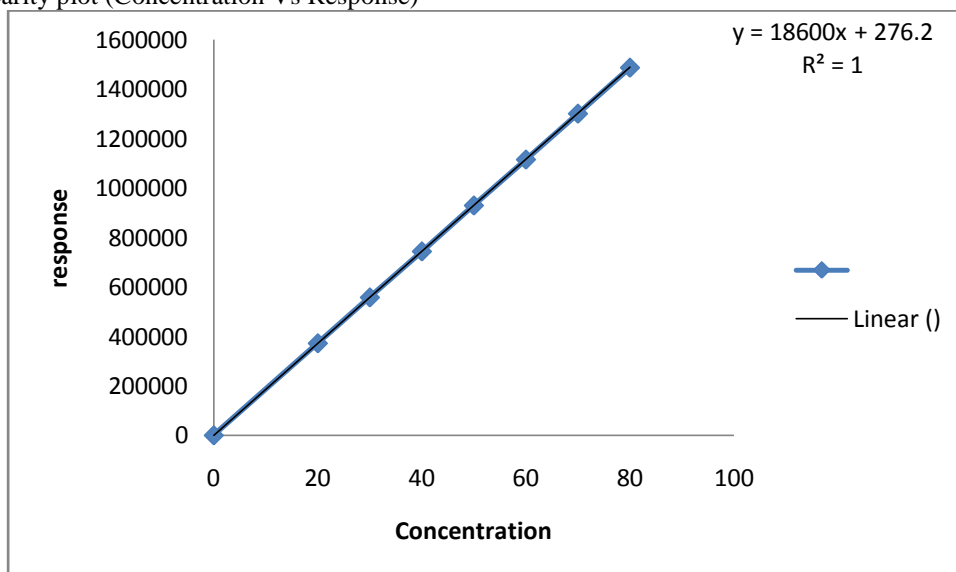
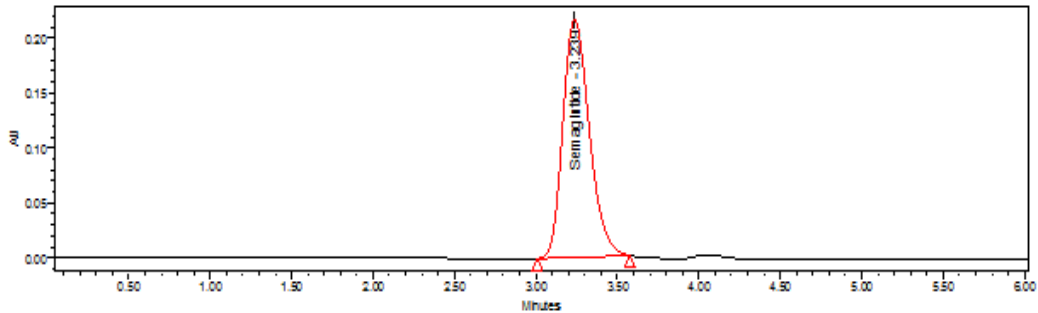
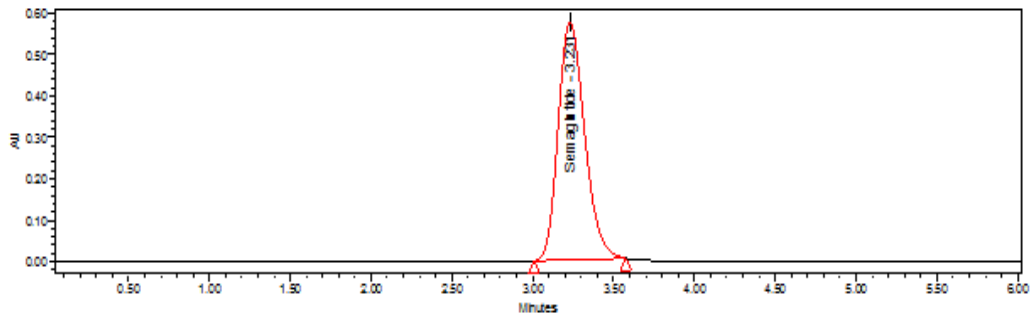


Fig:7.8.1 The chromatograms at 20 ppm are as follows:



Inference: The standard chromatogram of 20 ppm

Fig 7.8.4: There are chromatograms available. 70 parts per million



Inference: The standard chromatogram of 70 ppm

7.9 Ruggedly(Ruggedness):

Variability from system to system (System to System variability):

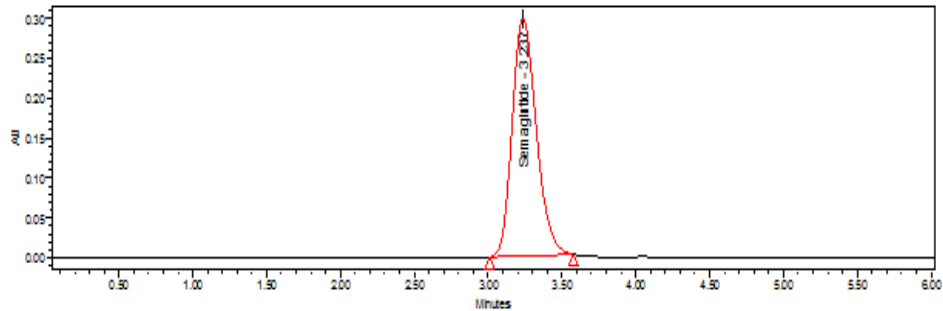
TABLEMENT: 7.9.1

Data on System Variability

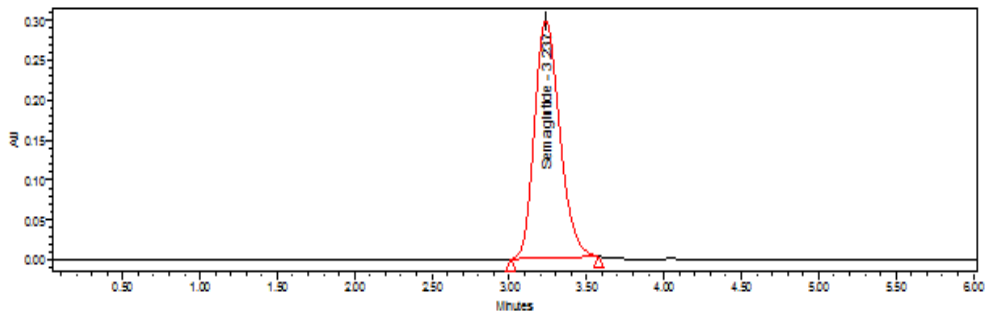
System-2

S.NO:	Peak area	Assay % of Semaglutide
1	634360	98.65
2	634098	98.63
3	635696	98.86
4	633289	98.52
5	634147	98.63
6	633495	98.55
Mean	634180.8	98.64
%RSD	0.019	0.12

Fig 7.9.1 System to system variability chromatograms



Inference: std- 1 chromatogram showing system-to-system variability



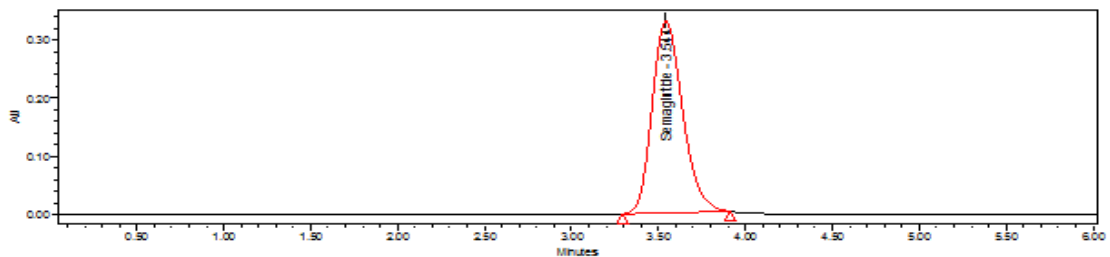
Inference: std- 2 chromatogram showing system-to-system variability

8. Resiliency (Robustness):

TABLE: 8.1 There's proof that flux rate variability has an impact

Flow	Std Area	Tailing factor	Flow	Std Area	Tailing factor	Flow	Std Area	Tailing factor
0.8 ml	620286	1.322089	1.0 ml	634322	1.604878	1.2 ml	602077	1.285372
	619282	1.331920		635792	1.584354		601854	1.319385
	621337	1.296438		634360	1.543805		602403	1.292055
	620456	1.315454		635696	1.568590		603421	1.304561
	620765	1.326551		633147	1.559986		602465	1.294621
Avg	620425	1.31849	Avg	634663.4	1.572323	Avg	602444	1.299199
SD	754.0018	0.013728	SD	1100.917	0.023367	SD	599.8833	0.013223
%RSD	0.086	1.04	%RSD	0.184	1.48	%RSD	0.09	1.01

Fig 8.1, Robustness chromatogram a) Variation in flow rate (for 0.8 ml/min flow) has an effect.



Inference: Standard for robustness chromatogram – 1

9. LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):  
From the linearity plot the LOD and LOQ are calculated:

$$\text{LOD} = \frac{3.3 \sigma}{S}$$
$$= \frac{3.3 \times 3244.904}{18600} = 0.57$$
$$\text{LOQ} = \frac{10 \sigma}{S}$$
$$= \frac{10 \times 3244.904}{18600} = 1.74 = 18600$$

#### IV. CONCLUSION

A simple, Accurate, precise method was developed for the Estimation of the Semaglutide in API form. Chromatogram was run through inertsil ODS C18 (250 x4.6mm, 5 $\mu$ ). Mobile phase containing Methanol : Water in the ratio 70:30 was pumped through column at a flow rate of 1.0 ml/min in the room temperature. Optimized wavelength selected was 274 nm. Retention time of Semaglutide was found to be 3.237mins . %RSD of the Semaglutide was found to be 0.9 . LOD, LOQ values obtained from regression equations of Semaglutide was 0.57 ,1.74 respectively. Regression equation of Semaglutide is  $y = 18599.8434x + 276.2281$ . Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Semaglutide, Methanol, RP-HPLC.

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