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Stability Indicating Assay Method (Siam) for Determination of Semaglutide by HPLC Using Qbd Approach

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ABSTRACT

An accurate, precise, robust and stability-indicating RP-HPLC method was developed for the estimation of Semaglutide using QbD approach. After conducting several trials using CCD method, one desirable method was optimized. Stationary phase selected was Kromasil C18 (250×4.6 mm, 5 μm) and potassium dihydrogen orthophosphate (pH_2) and Methanol used as mobile phase in the ratio of 61.2: 38.8. Detection was carried out at the wavelength 230nm. Flow rate selected for separation was 0.98ml/min and the temperature of 29.15^{0}C. The retention time was found to be 2.518 at the run time of 5min. The developed method was subjected to validation as per ICH guidelines. Semaglutide linear in the concentration range of 0-9μg/ml and the regression coefficient was found to be 0.999. The developed method was accurate, precise and robust. When a sample solution subjected to stress conditions like acid hydrolysis, neutral hydrolysis, photodegradation and thermal degradation, no degradation products were observed. In basic degradation studies two degradation products and oxidative conditions one degradation product was observed. From stress studies, we can observe that the degradation of Semaglutide was less than 10 in all conditions, and there is no impact of degradation products on system suitability parameters. The peak was homogenous I all conditions, thus proving the stability-indicating nature of the method. This method can be applied for the determination of Semaglutide in the drug substance.

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INTRODUCTION

SEMAGLUTIDE IUPAC name of Semaglutide is 17-{[(1R)-3-[(2-{2-[({2-[2-({[(5S)-5-[(2S) -2- [(2S) -2- [(2S) -2- {2- [(2S) -2- [(2S) -2- [(2S) -2- [(2S) -2-[(2S)-2-[(2S)-2-[(2S)-2-[(2S)-2-[(2S,3R)-2-[(2S)-2-[(2S,3R)-2-{2-[(2S)-2- {2-[(2S)-2 -amino-3- (1H-imidazol-4-yl) propanamido]-2- methyl propanamido} -4- carboxy butanamido] acetamido}-3-hydroxybutanamido]-3-phenylpropanamido]-3-hydroxy butanamido]-3-hydroxypropanamido]-3-carboxy propanamido]-3-methyl butanamido]-3-hydroxy propanamido] -3-hydroxyl propanamido] -3- (4-hydroxyphenyl) propanamido] -4- methyl pentanamido]-4-carboxybutanamido]acetamido}-4-carbamoyl butanamido] propanamido] propanamido] -5 -{[(1S) -1 -{[(1S) -1 -{[(1S,2S) -1 -{[(1S) -1- {[(1S) -1-{[(1S)-1-{[(1S)-1-{[(1S)-4-carbamimidamido-1-[({[(1S)-4-carbamimidamido-1-[(carboxymethyl) carbamoyl] butyl] car-

bamoyl}methyl) carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}-3-methylbutyl] carbamoyl}-2- (1H-indol-3-yl)ethyl]carbamoyl}ethyl]carbamoyl}-2-methylbutyl]carbamoyl}-2-phenylethyl]carbamoyl}-3-carboxypropyl] carbamoyl} pentyl] carbamoyl} methoxy) ethoxy]ethyl}carbamoyl)methoxy]ethoxy}ethyl) carbamoyl]-1-carboxy propyl] carbamoyl} heptadecanoic acid (Kuna *et al.*, 2019). Molecular weight 4113.641 and molecular formula is C187H291N45O59. Structure was shown in Figure 1.

QbD (Quality by Design) (Vogt and Kord, 2011; Peraman *et al.*, 2015) is a systemic approach used to produce the robust method, to increase patient safety by increasing the quality of pharmaceuticals (Raman *et al.*, 2015). In present days QbD approach is taking the predominant position in pharmaceutical studies. In analytical QbD approach, five main tools are present (Monks *et al.*, 2011; Orlandini *et al.*, 2013). Method goal identification, method scouting, method evaluation, method selection and risk assessment (Abdurrahman and Saxena, 2014).

Literature survey (Jadhav and Tambe, 2013; Bhatt and Rane, 2011) revealed that there are no studies on quality-based design approach of estimation of Semaglutide by HPLC method. A few HPLC studies were reported for estimation of Semaglutide (Kuna *et al.*, 2017). Few studies were reported on other QbD approach of other anti-diabetic drugs (Kalariya *et al.*, 2014; Karmarkar *et al.*, 2011). The pilgrimage has been made to develop a simple accurate, precise, rapid, economical, robust and stable method for determination Semaglutide by HPLC using the QbD approach according to ICH guidelines (Guideline, 2009).

MATERIALS AND METHODS

Chemicals and Reagents

Pure Semaglutide was procured from Spectrum pharma Pvt Ltd (Hyderabad). Hydrochloric acid AR grade (HCL) and sodium hydroxide AR grade (NaOH) were obtained from Merck India Pvt Ltd. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid AR grade was purchased from Fisher scientific, India and SD. Fine chem Ltd. Respectively. Ammonium acetate and ammonium formate were obtained from SD. Fine chem Ltd and Merck India Pvt Ltd. Respectively. HPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific. HPLC grade water used throughout analysis was obtained from the Merck milli-Q water purification unit.

Equipment

The LC system used for method development and validation. Waters carried detection with a diode array detector (model: 2996 detector 2487 separation module). The output signal was supervised and processed using Waters Empower 2 Software. Mettler Toledo balance was used to perform weighing. Received drug sample was authenticated by melting point apparatus (BUCHI), FT-IR (BRUKER ALFA), and UV-VIS spectrophotometer (Shimadzu -1800, japan). Other pieces of equipment used throughout the experimental work are hot air oven (Yorco scientific), thermostat dry air equipment Thermo scientific and pH meter (Eutech instruments pH tutor, pH meter, India).

Drug authentication

After procuring the API from the drug was authenticated by melting point test and scanning from 200 to 400nm in UV-VIS spectrophotometer.

Chromatographic Conditions

Various trials were conducted to select a mobile phase and a stationary phase. The choice of a Kromasil C18 column 0.1%OPA: Methanol (61.2:38.8%) was selected as mobile phase. Using the central composite design (CCD) method was optimized. CCD design summary was shown in Table 1.

Preparation of Solutions

Preparation of Standard Solution

Weigh accurately about 3mg of Semaglutide and dissolved in a diluent. The solution was sonicated for 5min and make up the volume to 50ml with diluent.

Preparation of buffer

Potassium dihydrogen orthophosphate buffer(pH_2)

Accurately weighed 1.36g of potassium orthophosphate was dissolved in 900ml milli-Q water and sonicate it for degassing. Makeup the volume to 1000ml and added 1ml of Triethylamine. Using diluted orthophosphoric acid pH was adjusted to 2.

Method Validation

The proposed method was validated as per ICH guidelines.

To conduct system suitability studies, six replicate samples of 6μg/mL solution of Semaglutide were injected into a system to calculate the retention time, area, theoretical plates, SD and %RSD. In linearity studies conducted by injecting different concentrations range of 1.5-9μg/ml. The calibration curve

was plotted by taking concentration on X-axis and peak area on Y-axis. The correlation coefficient (R^2) should be less than 1. Accuracy performed to determine percentage recovery by injecting 50%, 100% and 150% concentrations of standard in triplicate. The precision of the developed method was determined by two methods like repeatability (intra-day) and intermediate precision (inter-day). % RSD should be less than 2. Limit of detection (LOD) refers to the lowest concentration level resulting in the peak area of three times the baseline noise. Limit of Quantification (LO Q) refers to the lowest concentration level that provided a peak area with a signal to noise ratio higher than ten. Robustness referred as, the capacity of the method to remain unaffected by small or deliberate changes in chromatographic conditions like organic content in mobile phase ration (± 10), flow rate (± 10) and temperature (± 10).

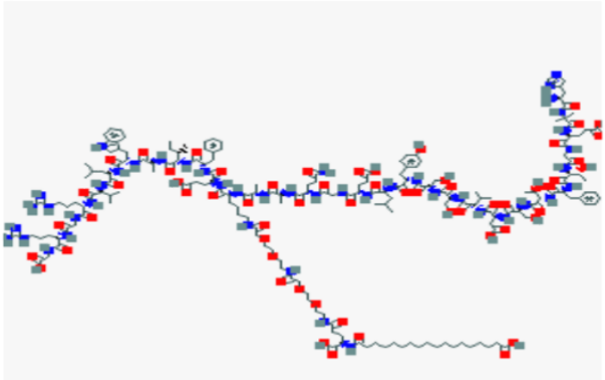


Figure 1: Semaglutide Structure

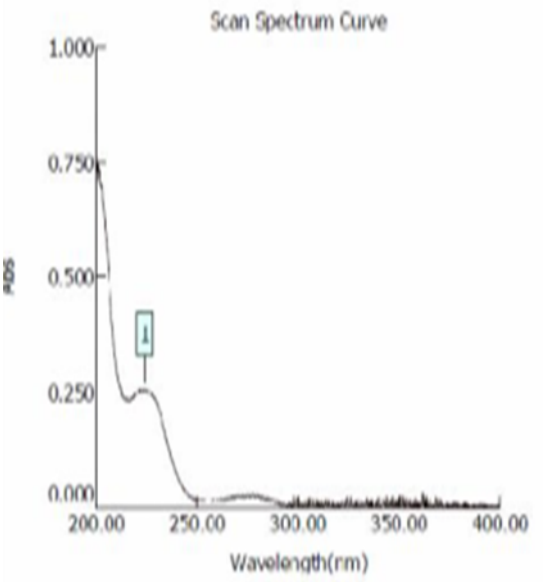


Figure 2: UV spectrum of Semaglutide

Stress Studies

To conduct acid hydrolysis, base hydrolysis and neutral hydrolysis, 1ml of stock solution added 1ml of 2N HCl, 1ml of stock solution and 1ml of 2N NaOH solution and 3mg of Semaglutide was weighed and dissolved in 1ml in individual volumetric flask respectively. Three solutions were shaken in Radley apparatus 70ºC for 1 hr then neutralized and diluted to 10ml. In oxidative degradation, to 1ml of stock solution, a 1ml of 20% H_2O_2 solution was added and kept in the dark area at room temperature for 24 hrs and diluted to 10ml. In thermal degradation 3mg of Semaglutide was kept in a petri dish and kept in a hot air oven at 70ºC for 24hrs. Sampling was done at multiple time points and dissolved in the diluent to produce 10ml. In photodegradation 3mg of Semaglutide was uniformly spread in the petri dish and exposed to sunlight for 24hrs. Sampling was done at multiple time points and dissolved in the diluent to produce 10ml.

RESULTS AND DISCUSSION

Drug authentication

After procuring the API from the drug was authenticated by melting point test and scanning from 200 to 400nm in UV-VIS spectrophotometer. The melting point was observed between 187-189^{0}C. From UV spectrum maximum absorption point found at 230nm in methanol. The UV spectrum was shown in Figure 2.

Method Development

Various trials were conducted to select the mobile phase and the stationary phase. The choice of Kromasil C18 column acetonitrile was selected and finalized as the organic modifier for further optimization study. Using the central composite design (CCD) method was optimized. The factors viz; % Organic concentration(33.3-46.7% Org %), flow rate (0.83-1.17ml/min), Column temperature (ranging from 24.95^{0}C and 35.05^{0}C) were taken and counter and a 3D surface plot showing the effect of each parameter on Retention Time, Theoretical plates and Asymmetry (CQA) were generated. Results and ANOVA studies were shown in Tables 2 and 3, respectively. A desirability function applied to the optimized conditions to predict retention time, asymmetry and theoretical plates.

2D contour plot was developed as a function of 1% organic concentration, pH and buffer strength. To understand the results, 2D contour plots and 3D plot were generated from data using Design Expert® software (shown in Figures 3 and 4).

To get an optimum set of conditions, composite desirability was applied based on the specified goals and limits of each response. If the response on the desirability scale is on 1, it is a thoroughly desirable

Table 1: Design Summary of CCD

File version: DX 11.0.0, Study Type: Response surface, Design Type: central composite Design, Design Model: Quadratic			ATP: Robustness CQA: Retention time, Theoretical plates and Asymmetry, Runs: 21		
CMPs	Unit	Type	Subtype	Min.	Max.
Column Temperature	0C	Numeric	Continuous	24.95	35.05
Flow rate	ml/min	Numeric	Continuous	0.8318	1.17
%Org ratio	%	Numeric	Continuous	33.27	46.73

Table 2: Central composite experimental design matrix with response

Std	Run	Factor 1 A: FR ml/min	Factor 2 B: MP %	Factor 3 C: T 0C	Response 1 RT MIN	Response 2 TP NUM	Response 3 TF NUM
6	1	1.1	36	33	2.465	5579.36	1.4
16	2	1	40	30	2.668	6038.26	1.44
15	3	1	40	30	2.671	5629.04	1.55
3	4	0.9	44	27	2.9	5511.93	1.44
7	5	0.9	44	33	2.891	5342.77	1.37
2	6	1.1	36	27	2.465	5760.58	1.39
20	7	1	40	30	2.67	5833.39	1.53
13	8	1	40	24.9546	2.655	6097.28	1.37
18	9	1	40	30	2.659	5666.54	1.45
10	10	1.16818	40	30	2.385	5335.45	1.41
12	11	1	46.7272	30	2.633	5759.36	1.44
4	12	1.1	44	27	2.447	5590.03	1.42
19	13	1	40	30	2.659	5534.37	1.46
9	14	0.831821	40	30	3.241	6262.6	1.37
5	15	0.9	36	33	2.911	5200.94	1.34
17	16	1	40	30	2.673	5890.92	1.4
1	17	0.9	36	27	3.026	5827.67	1.42
11	18	1	33.2728	30	2.606	4978.99	1.47
8	19	1.1	44	33	2.343	5450.19	1.26
14	20	1	40	35.0454	2.762	6052.56	1.51

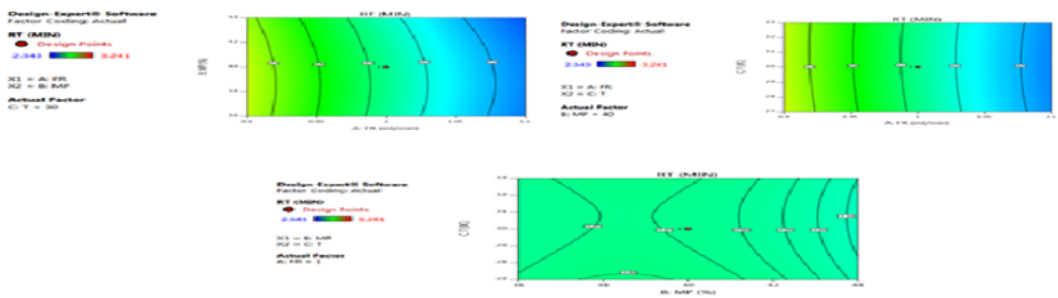


Figure 3: 2Dcontour plots of retention time as a function of FR, Column temperature and organic ratio

Table 3: ANOVA table for Retention time using CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.9153	9	0.1017	36.10	< 0.0001	Significant
A-FR	0.8703	1	0.8703	308.90	< 0.0001	
B-MP	0.0042	1	0.0042	1.50	0.2481	
C-T	0.0002	1	0.0002	0.0600	0.8115	
AB	4.500E-06	1	4.500E-06	0.0016	0.9689	
AC	0.0000	1	0.0000	0.0177	0.8967	
BC	5.000E-07	1	5.000E-07	0.0002	0.9896	
A^2	0.0282	1	0.0282	10.01	0.0101	
B^2	0.0084	1	0.0084	2.99	0.1146	
C^2	0.0008	1	0.0008	0.2722	0.6132	
Residual	0.0282	10	0.0028			
Lack of Fit	0.0280	5	0.0056	147.81	< 0.0001	Significant
Pure Error	0.0002	5	0.0000			
Cor Total	0.9435	19				

Table 4: Result of system suitability

S.No	RT	Area	Asymmetry	T. Plates
1	2.515	369765	1.38	4593
2	2.518	369582	1.4	4582
3	2.52	365226	1.44	4678
4	2.523	366412	1.42	4695
5	2.527	365262	1.43	4707
6	2.53	367302	1.42	4578
Mean	2.5	367258	1.4	4639
SD	0.01	2025.67	0.02	60.61
%RSD	0.2	0.6	1.5	1.3

Table 5: Result of linearity

Conc. (ppm)	Area
0	0
1.5	91489
3.0	183079
4.5	273992
6.0	364706
7.5	448744
9.0	546985

Table 6: Results for Accuracy

Value	50% (3μg/ml)	100% (6 μg/ml)	150% (9μg/ml)
1	543136	728383	905438
2	541102	728420	904090
3	546818	723033	906735
Mean	543685	726612	905421
SD	2897.3	3099.6	1322.6
%RSD	0.5	0.4	0.1
% Accuracy	99.5	100.2	99.7

Table 7: Results for Precision

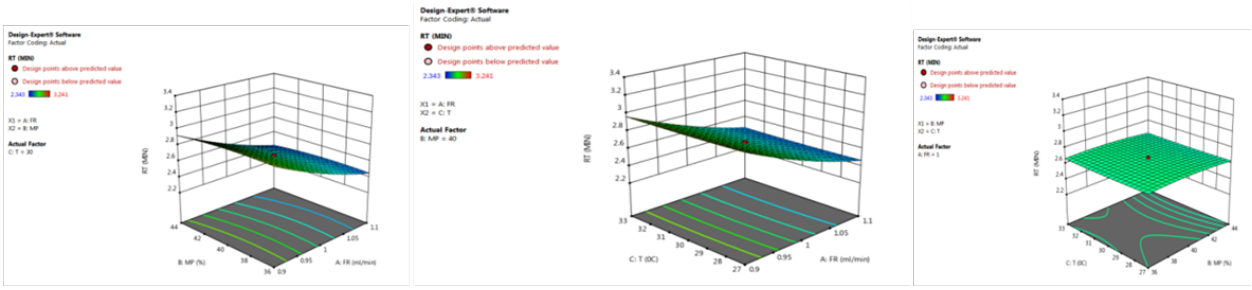
S.No	Intra-day precision	Inter-day precision
1	368190	345553
2	365689	341650
3	364421	344492
4	366696	340676
5	361043	339670
6	366406	337240
AVG	365408	341547
STDEV	2469.9	3084.3
%RSD	0.7	0.9

Table 8: Results of Robustness

Parameter	Modified	Area		% RSD	Asymmetry		%RSD
		Trial 1	Trial 2		Trial 1	Trial 2	
Flow rate	0.9	332821	327419	1.16	1.37	1.4	1.53
	1.0	369765	369582	0.04	1.38	1.4	1.02
	1.1	383068	388710	1.03	1.5	1.49	0.47
Column temperature	250C	365079	362042	0.59	1.38	1.4	1.02
	300C	365226	366412	0.23	1.44	1.42	0.99
	350C	331291	330928	0.08	1.5	1.48	0.95
% Org ratio	36.0	376493	378468	0.37	1.42	1.40	1.00
	39.9	368190	365689	0.48	1.41	1.39	1.01
	42.0	358893	360045	0.23	1.38	1.35	1.55

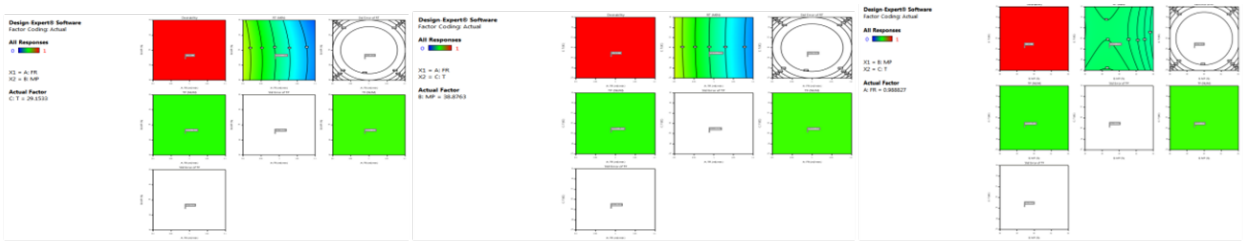
Table 9: Summary of degradation study

S. No.	Condition of degradation study	% of drug degraded	The retention time of degradant
1.	2N HCl, 8 hrs	4.87%	-
2.	2N NaOH, 8hrs	5.67%	-
3.	Neutral hydrolysis, 24 hrs	No degradation	-
4.	Oxidative degradation, 24 hrs	7.26%	2.254
5.	Thermal degradation, 3 days	3.16%	-
6.	Photodegradation, 24 hrs	1.77%	-



(a) (b) (c)

Figure 4: 3D contour plots of retention time as a function of FR (a), Column temperature (b)and organic ratio(c)



(a) (b) (c)

Figure 5: Overall desirability of the final method

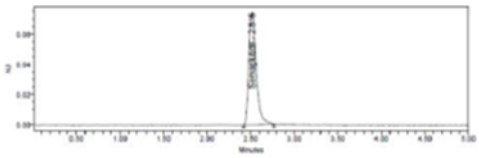


Figure 6: Optimized chromatogram of Semaglutide

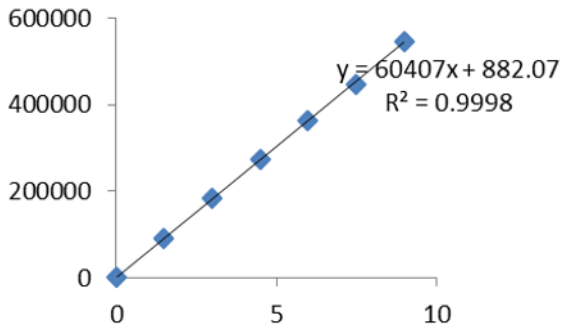


Figure 7: Linearity plot of Semaglutide

response, and response is on 0 it is an undesirable response. Responses based on specified goals and boundaries for retention time, area, and asymmetry obtained desirability composite was 1. Shown in Figure 5.

Optimization of Chromatographic Conditions

Initial trials were conducted to optimize the method according to the central composite design (CCD) method. HPLC studies were carried out using a Kromasil C18 (250×4.6 mm, 5 μm) and 0.1%OPA: Methanol (61.2:38.8%) as mobile phase. At wavelength detection of 230nm. The flow rate was selected as 0.98ml/min at 29.15^{0}C column temperature. The retention time was found to be 2.518min. The optimized chromatogram is shown in Figure 6.

Method Validation

The optimized method was subjected to method validation as per ICH guidelines. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics.

System Suitability

After injecting six replicate samples of 6μg/mL solution of Semaglutide into the system. Different parameters like retention time, area, theoretical plates, SD and %RSD were calculated. All the parameters were within limits as per guidelines. Results were shown in Table 4.

Linearity

The regression equation obtained was Y=60407x+0. r^2 (Correlation co-efficient) was found to be 0.999. Results were shown in Figure 7 and Table 5.

Accuracy

The accuracy of Semaglutide showed good recovery at each level and mean peak area and standard deviation are given in Table 6.

Precision

The % RSD of intraday and inter-day precision were found be 0.7 and 0.9, respectively. The results were within limits as per the guidelines and shown in the Table 7.

Limit of Detection (LOD) and Limit of Quantitation (LO Q)

LOD and LOQ were calculated according to the signal to noise ratio method. The LOD and LOQ obtained were 0.019 and 0.056 μg /mL, respectively.

Robustness

Robustness studies were performed by altering the parameters like column temperature, flow rate and % organic concentration. The % RSD was calculated and found that the results within limits. Results were shown in Table 8.

Stress Studies

Stress degradation studies have been performed for the drug by using various stress conditions. No degradation products were found in case of acid hydrolysis, neutral hydrolysis, photodegradation and thermal degradation. Two significant degradation products were found in the case of 2N base degradation, and one degradation product was found in 10% H2O2. The results were showing satisfactory, that is % degradation should be less than

10% and should not affect system suitability parameters of Semaglutide. Results were shown in Table 9.

CONCLUSIONS

A simple analytical and robust HPLC method was developed for the determination of Semaglutide by using the QbD approach using Design Expert® software. Results which were obtained from the validation of the developed analytical method were within the limit as per ICH guidelines. Validated stability-indicating HPLC method for Semaglutide was developed, which is capable of separating drug substance from the degradation products.

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Conflict Of Interest

The authors have no conflict of interests to disclose other than what has been acknowledged above.

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