INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Method Development of Semaglutide And Its Application for Bioanalysis of Clinical Study Samples for Pharmacokinetic Outcome

V. Bhosale, D. Bagde, V. Deshmukh, V. Pukale, R. Kapse, S. Bhakare, S. Salunkhe, A. Mokate, N. Gangurde

Bioanalytical Department, Bion Clinicals Pvt Ltd, Pune, India

ARTICLE INFO

Published: 18 Jan. 2025
Keywords:
ICH M10, Bioanalytical Method, Buffer Solution, HPLC Grade Water, analyte RT, Sensitivity, Sonicator.
DOI:
10.5281/zenodo.14685172

ABSTRACT

Our study on developing a Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for quantifying Semaglutide in human plasma using LC-MS/MS sounds meticulous and comprehensive. Chromatographic conditions where Column: C18 used, column Oven Temperature: 70ºC. Mobile Phases Prepared Pump-A : 0.1% formic acid in acetonitrile, Pump-B: 10 mM ammonium acetate with 0.1% formic acid in water. Linearity: Regression value of 0.998, % RSD of Repeatability and Precision: ±15%.During development. We have Challenges for Carryover Effect during development and 100% carryover observed in normal isocratic and binary flow methods. To reducing the carryover in method, we have developed a binary flow method in HPLC with gradient adjustment from 5% to 90% organic phase, where we have observed reducing carryover and improving LLOQ signal in development phase. Also, we have studied for ionisation Issues with Semaglutide for Improper ionisation at lower concentrations (1.000 ng/mL and 2.000 ng/mL) and when we have modified the mobile phase 01% formic acid in water to 2 mm ammonium acetate in water, where good ionisation and retention of Semaglutide was observed. The method was found to be simple, precise, accurate and validated according to ICH M10 guidelines1 for Pharmacokinetics evaluation of clinical samples.

INTRODUCTION

Semaglutide is an anti-obesity drug used for long-term weight control and an antidiabetic drug used to treat Type 2 Diabetes.2 It is a peptide with a side chain that resembles the hormone glucagon-like peptide-1 (GLP-1). It can be taken orally or delivered via subcutaneous injection.

MATERIALS AND METHODS:

Bioanalytical Method Development

Bioanalytical method development is the process of creating and optimizing a procedure to identify and quantify an analyte (drug) in human plasma. The goal is to ensure accurate, précises and

***Corresponding Author:** V. Bhosale
Address: *Head Bioanalytical Department, Bion Clinicals Pvt Ltd, Pune, India.*
Email ✉: vinayak@bionclinicals.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

reproducible measurements for a range of compounds, especially those that are novel or unknown.During method development of Semaglutide, sample processing and analysis has involved several key considerations:

Semaglutide has polar compound and its molecular weight 4114 g/mol, highly soluble aqueous phase.3 Challenges for Sample preparation: Semaglutide is unable to extract from human plasma at 4000 rpm centrifugation. It has protein binding around 99% to human plasma.

Solution: Extraction of Semaglutide from human plasma organic solvent of methanol was used and centrifuge samples around 15000 rpm. After centrifugation we observed accurate recovery around 60% Semaglutide from human plasma. Hence, we have developed a method of Protein precipitation for samples extraction, which will be useful for estimation of Semaglutide in human plasma by LC-MS/MS and evaluation of bioequivalence.4

Chemicals and Reagents: Reagents and Materials

1. Acetonitrile
2. Ammonium Acetate
3. Autosampler Vial and Caps
4. Dimethyl Sulphoxide
5. Formic Acid
6. HPLC Grade Water
7. K2EDTA human plasma
8. MAX Cartridge (Waters)
9. Methanol
10. Micro Centrifuge Tube
11. Ria Vial and Cap
12. Semaglutide
13. Semaglutide D8

Procedure during development :

Preparation of Solutions

Preparation of standard solution of Semaglutide

Semaglutide Stock Solution Weigh an about Semaglutide Working Standard which is Equivalent to 1.000 mg of and transfer into a 1.000 mL volumetric flask. Dissolve it in 0.200 mL of DMSO and added the 1% formic acid make up the volume make up to the mark with the Acetonitrile to produce a solution of 1 mg/mL of concentration of Semaglutide. Store the Stock solution at 2-8℃ storage condition.

Semaglutide D8 Stock Solution

Weigh an about Semaglutide D8 Working Standard which is Equivalent to 1.000 mg of and transfer into a 1.000 mL volumetric flask. Dissolve it in 0.200 mL of DMSO and added the 1% formic acid make up the volume make up to the mark with the Acetonitrile to produce a solution of 1 mg/mL of concentration of Semaglutide D5. Store the Stock solution at 2-8℃ storage condition.

0.1%Formic Acid Solution (BUF-A-DD/MM)

Take 500 ml of HPLC grade water and transfer to 1000 ml volumetric flask, added 1 ml Formic Acid and made up to the mark and mix well.

Diluent Solution 1(Acetonitrile : HPLC Grade Water:: 50:50)

Take 500 ml of Acetonitrile and 500 ml of HPLC grade water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.

Reconstitution Solution : (Acetonitrile : 0.1%Formic Acid Solution:: 75:25 v/v)

Take 750 ml of Buffer Solution (Acetonitrile: HPLC grade Water: 50:50 v/v) and 250 ml of 0.1%Formic Acid Solution by using measuring cylinder and transfer into a 1000 ml of reagent bottle, mix well and degas in sonicator and keep at room temperature.

Rinsing Solution (Acetonitrile : HPLC Grade Water: 70:30, v/v)

Take 700 ml of Acetonitrile and 300 ml of HPLC grade water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.

Diluent Solution 2 (Methanol : HPLC Grade Water: 50:50, v/v)

Take 500 ml of Methanol and 500 ml of HPLC grade water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.

Organic Mixture (Acetonitrile: Methanol, 50:50 v/v)

Take 500 ml of Acetonitrile and 500 ml of Methanol by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.

10mM ammonium Acetate in HPLC water

Weigh about 0.7708 g of Ammonium Acetate and transfer to 1000 ml volumetric flask, dissolve in HPLC water and volume made up to the mark with the HPLC water and mix well.

Chromatographic conditions

Polarity : Positive
Column : C18 100
Mobile Phase : Pump A- 0.1 % Formic Acid Solution in acetonitrile v/v
Pump B- 0.1 % Formic Acid in 10 mm Ammonium Acetate Solution in water v/v
Injection Volume : 25 µL
Flow rate : 0.3000 ml/minute
Column Oven temperature: 70 ℃
Sample Cooler temperature: 5℃
Expected RT : Semaglutide : 8.02 minutes (±0.5 minutes)
Semaglutide D8 : 8.02 minutes (±0.5 minutes)
Run time : 12.00 min

MRM transition for Semaglutide and Semaglutide D8 in Mass Spectrometry:

Detector Conditions:

Ion Source	Turbo Spray
Polarity	Positive
Resolution Q1	Unit
Resolution Q3	Unit
CUR	25.00
CAD	12.00
IS	5500.00
TEM	550.00
GS1	50.00
GS2	70.00

Analyte/IS Name	Q1	Q3	Dwell time	DP	EP	CE	CXP
Semaglutide	1029.300	1238.300	300.00	110.00	10.00	46.00	15.000
		1302.900	300.00	110.00	10.00	46.00	15.000
Semaglutide D8	1031.400	1238.500	200.00	110.00	10.00	46.00	15.000
		1303.800	200.00	110.00	10.00	46.00	15.000

Experiment For Extraction Trial-I in Human Plasma :

Extraction Trial 1 for protein Precipitation Method

Retrieve the required set of K_2ETA human plasma samples from the deep freezer and allow them to thaw at room temperature and arrange the sample as per trial samples.
Vortex the thawed samples to ensure complete mixing of contents.
Aliquot 380 µL of Plasma sample and then added 20 µL that is 5% of Working solution of Semaglutide in to pre labelled Ria vial.
Add 50 µl of internal standard dilution solution (Semaglutide D8) to each pre labelled Ria vial except blank sample and add 50 µL of diluent solution in blank sample vortex for few second.
Add 0.900 ml of Methanol and cap all the samples.
Vortex all samples for 10 min and 2500 rpm on vortexer.
Keep all samples for Centrifugation for 10 minutes at 5°C and 15000 rpm in a refrigerated centrifuge.
Separate-out the Surprenant of 0.800 mL of methanol from the centrifugated samples into separate ria vial and then evaporate the samples at 45°C in nitrogen evaporator.
Reconstitute the evaporated samples with 0.200 ml of mobile phase and transfer into the autosampler vials for analysis.

Evolution of Trial-I :

Recovery of Semaglutide was found exact 64% and linearity was successfully passed, regression was more than 0.98. Precision of the trial samples was less than 15%

Extraction Trial II for Solid Phase Extraction

Retrieve the required set of K_2ETA human plasma samples from the deep freezer and allow them to thaw at room temperature and arrange the sample as per trial samples.
Vortex the thawed samples to ensure complete mixing of contents.
Aliquot 380 µL of Plasma sample and then added the 20 µL that is 5% of Working solution of Semaglutide in to pre labelled Ria vial.
Add 50 µl of internal standard dilution solution for Semaglutide D8 to each pre labelled Ria vial except blank sample and add 50 µL of diluent solution in blank sample vortex for few second.
Add 0.900 ml of Methanol and cap all the samples.
Vortex all samples for 10 min and 2500 rpm on vortexed.
Keep all samples for Centrifugation for 10 minutes at 5°C and 15000 rpm in a refrigerated centrifuge.
Use SPE method, Waters MAX SPE 30 mg/mL Cartridges.
Conditioning: - 1.000 mL of methanol Equilibration: - 1.000 mL of HPLC Grade Water
Load the entire Surprenant from the samples on Cartridges.

Washing step, I: 1.000 mL of 10 mM Ammonium Acetate Solution.
Washing step II: 1.000 mL of Methanol.
Elute all samples with 1.000 mL of Methanol: acetonitrile 50 :50 v/v.
Dry all samples on nitrogen Evaporator at 40 °C until dryness.
Reconstitute all samples with 0.200 ml Reconstitution Solution and vortex for few second.
Transfer the samples into pre labelled auto sampler vials for analysis.

Evolution of Trial-II :

Recovery of Semaglutide was found around 80.54% and linearity was successfully passed, regression was more than 0.98. Precision of the trial samples was less than 10%.Based on results of above trial 1 and 2 we have decided and more accurate and cost-effective method is Protein precipitation and same method was used to evaluate the following experiments;

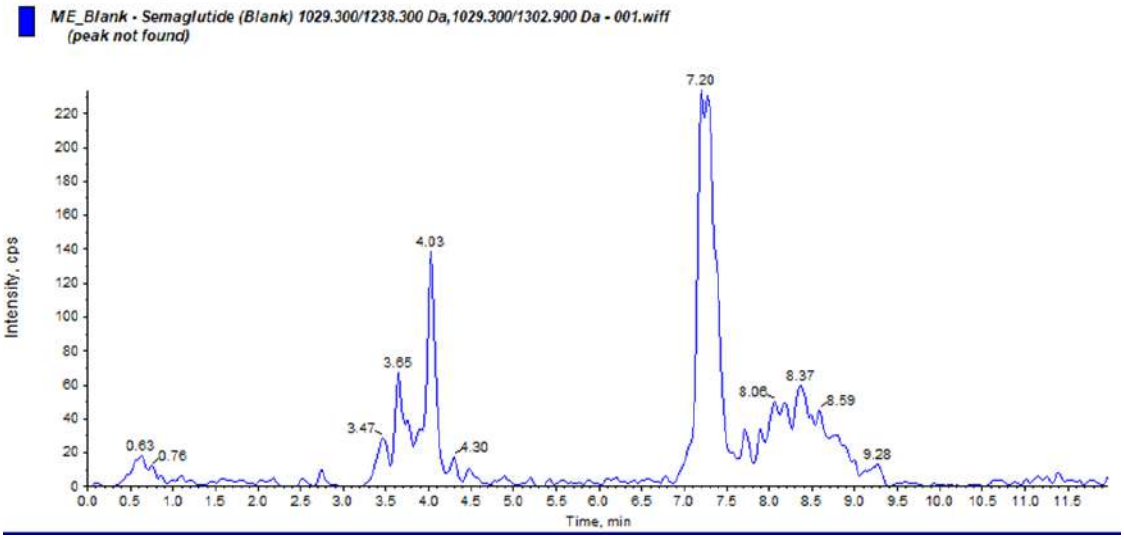
RESULTS AND DISCUSSION :

Experiment Name	Observed Results	
	ICH M10 Acceptance Limit	**Semaglutide**
Selectivity	Interference Should be less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response in all six different lot of human plasma samples	Interference was observed less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response in all 10 different lot of human plasma samples
Matrix Effect	Coefficient of variation Should be ≤ 15 % Nominal value of QC ±15%	QC Coefficient of variation: 5.87 to 10.48% QC % Nominal value 91.610 to 114.508%
Specificity	Interference should be less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response	Interference was observed than 0.00% at analyte RT and less than 0.36% at IS RT with respective LLOQ area response
Sensitivity	Mean % Nominal: 100; ±15% CV: 5.87% Coefficient of variation Should be ≤ 20.00 Signal to noise ratio should be ≥ 5.00.	Mean % Nominal: 103.835 % CV: 5.57% Signal to noise ratio was more than 5 and average for all samples was 59.803.
Precision and Accuracy (Quality Control Samples)	Coefficient of variation Should be ≥15 % Nominal value of QC ±15%	Coefficient of variation: 2.83 to 9.94% % Nominal value 91.622to 107.661 %
Recovery	Coefficient of variation Should be ≥15 at all level	Mean Analyte Recovery: 71.990% Mean Internal Standard Recovery: 71.123%
Carryover	Carryover should be less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response	Interference was observed than 0.00% at analyte RT and less than 0.00% at IS RT with respective LLOQ area response

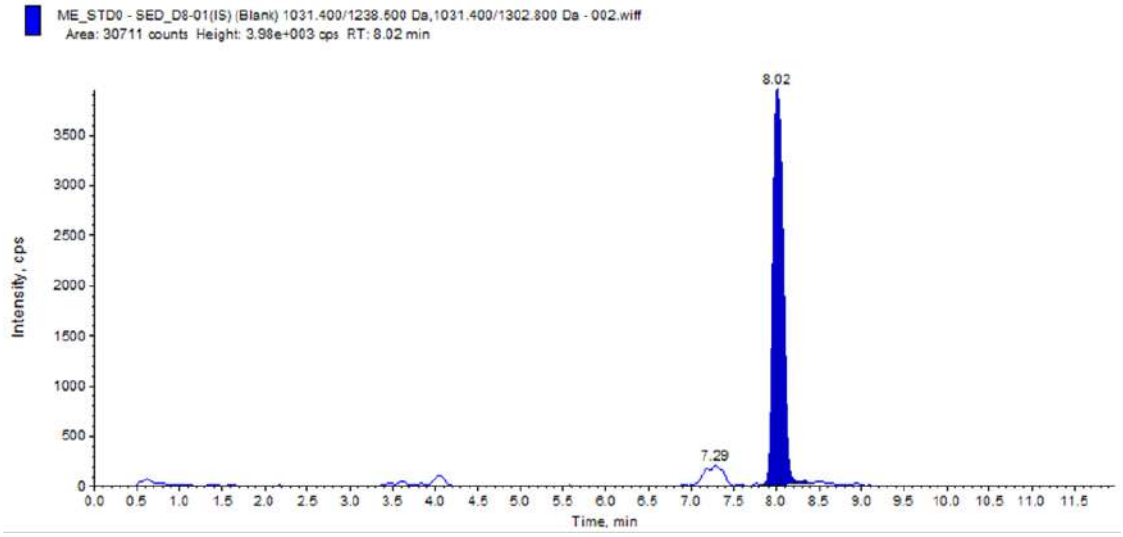
Results of LC-MS/MS

Representative Chromatogram of Semaglutide

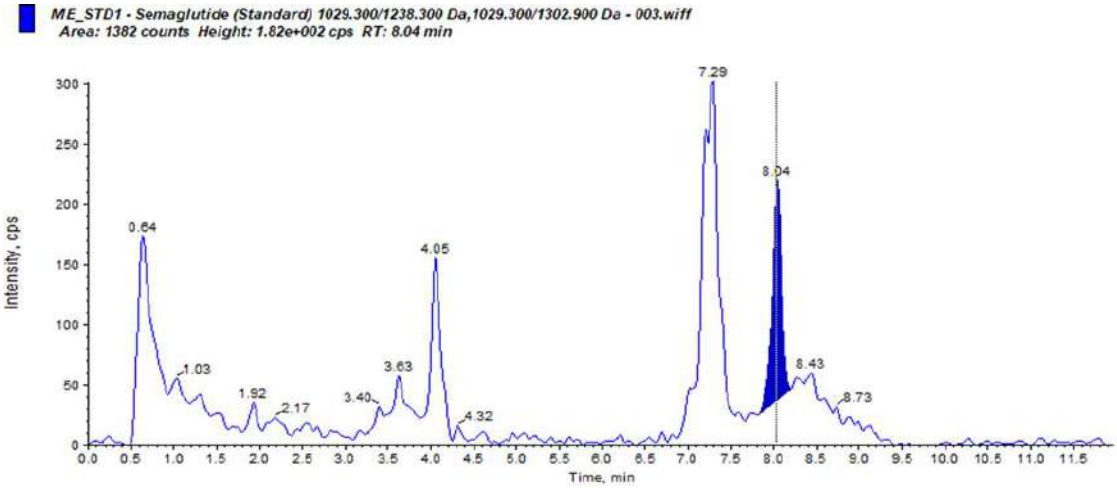
Blank for Semaglutide



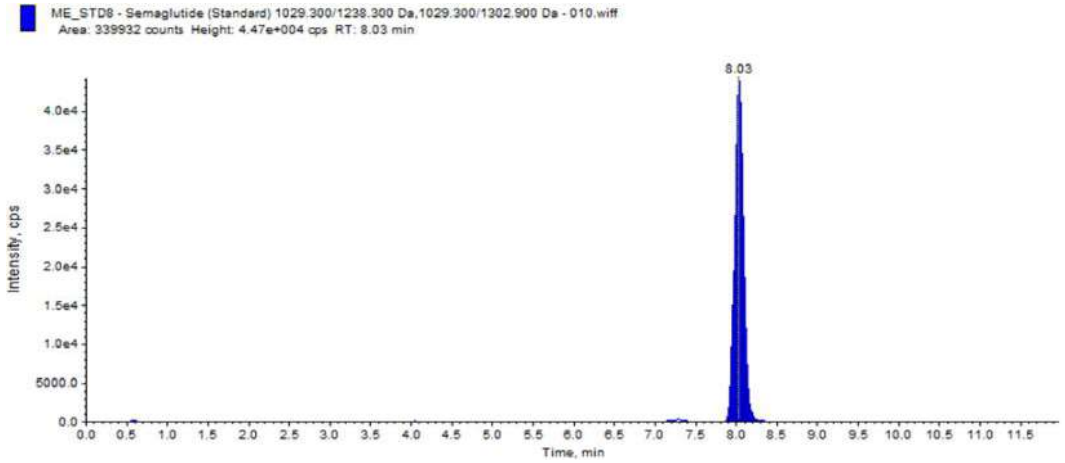
2. Semagalutide D8 (Std 0)



3. LLOQ (1.000 ng/mL) Std I for Semaglutide



4. ULOQ (200.000 ng/mL) Std VIII for Semaglutide



Ethical Considerations :

This study has been conducted after Institutional Ethics Committee approval.

Informed consents obtained from the study participants as per regulatory guidelines.

Financial Support and Sponsorship :

Nil.

Conflicts of Interest :

There are no conflicts of interests

REFERENCES

1. ICH M10 on bioanalytical method validation - Scientific guideline | European Medicines Agency (EMA)
2. Xingbang Liu, Na Zhang, Xiaotong Gu, Yinhui Qin, Di Song, Long Zhang, Shutao Ma, Total Synthesis of Semaglutide Based on A Soluble Hydrophobic-Support-Assisted Liquid-Phase Synthetic Method, ACS Combinatorial Science, October 15, 2020.
3. https://pubchem.ncbi.nlm.nih.gov/compound/Semaglutide#section=Chemical-and-Physical-Properties, Pubchem
4. https://www.accessdata.fda.gov/drugsatfda_docs/psg/PSG_213051.pdf, Draft Guidance on Semaglutide, August 2021

HOW TO CITE: V. Bhosale, D. Bagde, V. Deshmukh, V. Pukale, R. Kapse, S. Bhakare, S. Salunkhe, A. Mokate, N. Gangurde, Method Development of Semaglutide And Its Application for Bioanalysis of Clinical Study Samples for Pharmacokinetic Outcome, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 1, 1442-1448. https://doi.org/10.5281/zenodo.14685172