Automated SPE Sample Preparation for Bioanalysis of GLP-1 Analogs from Plasma

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INTRODUCTION

Glucagon-Like Peptide 1 (GLP-1) analogs, initially intended to treat type II diabetes Mellitus, are currently used for obesity and cardiovascular health management. In addition, investigation is underway for neurodegenerative and cognitive disorders. To fast-track these investigations and assess drug efficacy, safety, immunogenicity and metabolism, robust, selective and sensitive analytical methods are needed. Because these synthetic analogs are modified to resist enzymatic degradation and increase duration of action in vivo, pharmacokinetic studies of therapeutic peptides are complicated and can present challenges in sample preparation and quantification. Achieving high recovery, reproducibility, and balancing throughput while minimizing matrix effects is critical for accurate pharmacokinetic (PK) and metabolism studies.

EXPERIMENTAL

Exenatide Acetate (AA0039QE), Liraglutide (AA003R7S), Lixisenatide (AA00C6IL), Tirzepatide (AA01MVAI) and Semaglutide (AA00H208) from aablocks were dissolved in diluted Methanol. 200µL of Sprague Dawley Rat plasma was spiked before precipitation with 200µL of cold, acidified Acetonitrile. The supernatant was then diluted with 400µL of acidified water and loaded onto Oasis™ PRiME HLB µElution™ Plate (Waters, P/N 186008052). After eluting 2x with 25µL 3% ammonium hydroxide in 75% acetonitrile (summarized in **Figure 1**), eluates were diluted 1:1 with water and directly injected to ACQUITY™ Premier System paired with Waters Xevo™ TQ Absolute MS. QuanRecovery™ Collection Plates were used to minimize non-specific binding.
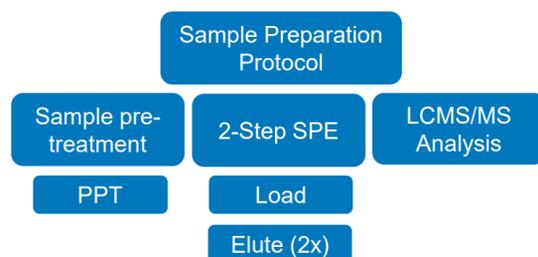
Figure 1: *Representation of sample preparation protocol: spiked plasma is pre-treated with Acetonitrile and GLP-1 analogs are purified in a 2-step SPE.*

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        Sample Preparation
            Protocol

Sample pre-    2-Step SPE    LCMS/MS
treatment                    Analysis

  PPT            Load

              Elute (2x)
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LC Conditions:
System: ACQUITY Premier System with FTN
Column: ACQUITY Premier Peptide CSH™ C18 Column, 130Å, 1.7 µm, 2.1 x 50 mm (Waters, P/N 186009487)
Column Temperature: 65°C
Mobile phase A: 0.1% Formic Acid in 18.2MΩ*cm water (v/v)
Mobile phase B: 0.1% Formic Acid in Acetonitrile (v/v)
Injection volume: 10 µL
Weak Needle Wash: 0.1% Formic Acid in Acetonitrile (v/v)
Strong Needle Wash: 10% Acetonitrile in 1% Formic Acid (v/v)

Time (min)	Flow (mL/min)	% A	%B	Curve
Initial	0.4	100	0	Initial
0.25	0.4	100	0	6
0.75	0.4	25	75	6
1.5	0.4	0	100	6
4.25	0.4	0	100	6
4.5	0.4	100	0	6
5	0.4	100	0	6

MS Conditions:
System: Xevo TQ Absolute MS
Ionization mode: ESI positive
Capillary Voltage: 2.00 kV
Cone Voltage: 32 V
Desolvation temp.: 500°C
Desolvation gas flow: 1100 L/Hr
Cone Gas Flow: 150 L/Hr
MS Software: MassLynx™ 4.2 Software, TargetLynx™ 4.2 Software

Automation and Smart Devices:

Andrew+™ Robot, Pipette+™, and Extraction+™ Connected Devices driven by OneLab™ Software.

Compound	Precursor (m/z)	Product (m/z)	Cone (V)	Collision (eV)
Exenatide Acetate	687.222	159.1149	32	35
Exenatide Acetate	687.222	237.1534	32	35
Lixisenatide	608.1398	601.1023	40	10
Lixisenatide	608.1398	605.774	40	10
Liraglutide	938.68	135.8	40	41
Liraglutide	938.68	1064.08	40	22
Semaglutide	1029.3	1238.1	46	32
Semaglutide	1029.3	1302.9	46	32
Tirzepatide	1204.239	396.29	10	32

RESULTS

Automation of the purification of 5 GLP-1 analogs (Semaglutide, Exenatide, Liraglutide, Lixisenatide and Tirzepatide) from plasma for LC-MS/MS quantification was achieved using the Andrew+ Robot, Pipette+ and Extraction+ Connected Devices with OneLab Software. The 5 analytes were reproducibly extracted and quantified using the described experimental method.
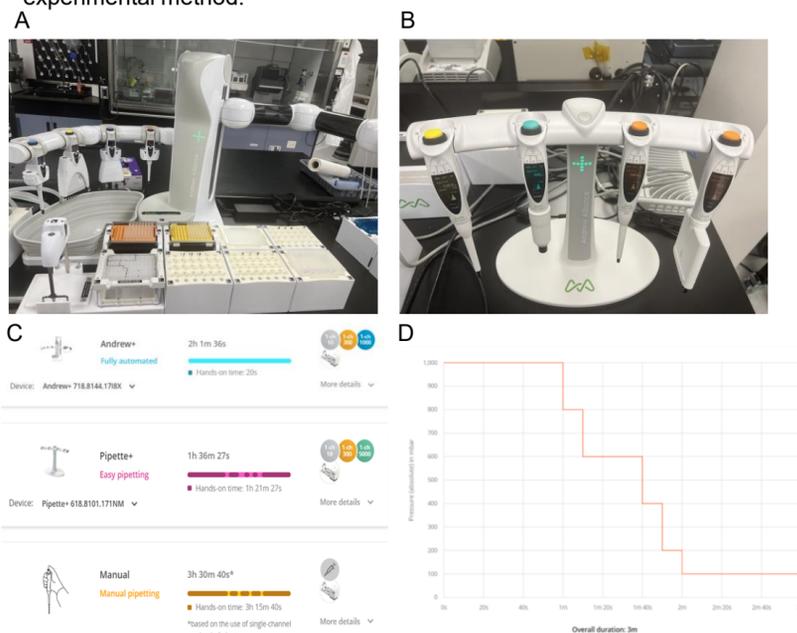

Figure 2: *Andrew+ Robot Fully automated (A) and Pipette+ Connected Device Easy pipetting (B) setup for GLP-1 analogs extraction. Figure 2C compares the amount of time consumed to execute the procedure fully automated vs. assisted pipetting vs. fully manual operation. A 3-minute pressure gradient used on the Extraction+ Connected Device is shown in Figure 2D.*

The workflow was evaluated fully manually to first determine suitability, then fully automated with Andrew+ Robot and Extraction+ Connected Device, and an assisted approach with Pipette+ and Extraction+ Connected Devices. Depending on the approach used, the total time of the workflow is varied. As seen in **Figure 2C**, a fully manual approach will take 3h30min, while moving to a fully automated workflow reduces time and manual user input at 2h1min. A middle-ground is using Pipette+ Connected Device Easy pipetting, 1h36min. Use of OneLab Software allows for all workflows to be fully traceable for compliance.

Both fully automated and assisted approaches resulted in calibration curves between 5-1000 ng/mL with R² values > 0.99 (**Figure 3**) across all 5 analogs.
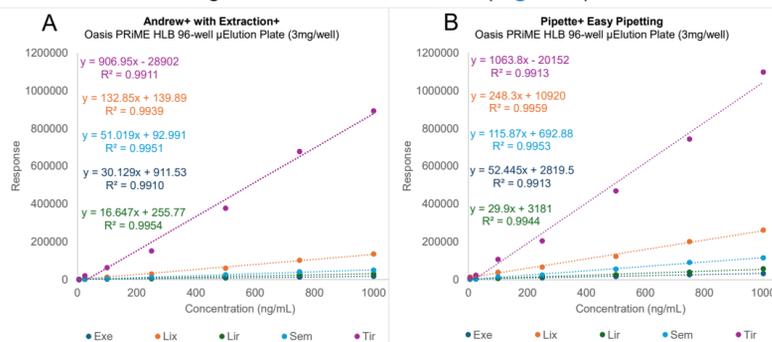

Figure 3: *Andrew+ Robot Fully automated (A) and Pipette+ Connected Device Easy pipetting (B) calibration for GLP-1 analogs extraction.*

The sample preparation approach was optimized as various precipitation conditions were evaluated prior to SPE using Oasis PRiME HLB µElution Plate and produced low matrix effects (ME%) and consistent recoveries across all three approaches, showing suitability for GLP-1 extraction in bioanalysis. Oasis PRiME HLB µElution Plate was chosen due to its fast 2- and 3-step protocols and additional cleanup of phospholipids applicable for bioanalysis.

Analyte	ME (%)
Exenatide	13.8
Lixisenatide	-16.4
Liraglutide	19.5
Semaglutide	-18.5
Tirzepatide	-8.8

DISCUSSION

As demand for reproducible and reliable data increases, many are turning to automation. Automation offers accurate and reproducible data, higher throughput and faster turnaround, eliminates human bias and error, enhances compliance and traceability and provides efficient workflows that garner long-term cost savings.

This approach shows automated SPE coupled with LC-MS/MS can be successfully applied to quantify diverse GLP-1 peptides in plasma. In this work, the fully automated approach encountered some challenges due the required solvents used in elution, leading to evaporating and poor recovery. In an optimal workflow, temperature needs to be strictly controlled to reduce this risk. The Andrew+ Robot family does include a device with temperature control called Peltier+ Connected Device; however, volumes would need to be rescaled to be amenable. To reduce risk of evaporation, the assisted pipetting with Pipette+ Connected Device reduces the workflow time by ~0.5 hours. When taking these considerations, both approaches can be suitable for GLP-1 extraction.

In this work, Oasis PRiME HLB µElution Plate was used as previous work tried Oasis HLB µElution Plate. Oasis PRiME HLB µElution Plate is a general-purpose sorbent with broad applicability in bioanalysis, but future Waters work will examine how other Oasis Sorbents may improve recovery for GLP-1s. Additional future work will involve more automated workflows for small molecules and peptides with the Andrew+ Robot platform.

Figure 4: *Extraction+ Connected Device.*

CONCLUSIONS

- OneLab Software offers audit-trailed, cGLP-compliant software that provides easily integrated and validated protocols

- Simple and quick 2-step SPE protocol using Oasis PRiME HLB µElution Plate sample plates achieved reproducible recoveries of Semaglutide, Exenatide, Liraglutide, Lixisenatide and Tirzepatide from rat plasma

- QuanRecovery Collection Plates mitigate non-specific binding, improving recovery

- Clean, low matrix effects (<20%) for all 5 analytes using Oasis PRiME HLB µElution Plate

- Whole protocol executed with Andrew+ Robot fully automated is ~1.5 hours less than manual execution, and assisted, easy Pipette+ Connected Device is ~2 hours less than manual extraction (**Figure 2C**)

- Linear calibration curves in the range of 5-1000ng/mL

- Automation enables faster and better data, lower risks (analyst-related errors), higher compliance and long-term cost savings

References

1. M. Trudeau and A. Scumaci, SPE-LC/MS Bioanalytical Quantification of the Biotherapeutic Peptide, Semaglutide from Plasma, (2023). Waters Application Note: 720008097.
2. T.S. Lee et al., Novel LC-MS/MS analysis of the GLP-1 analog semaglutide with its application to pharmacokinetics and brain distribution studies in rats, Journal of Chromatography B, 1221 (2023), 123688.
3. Y. Kim et al., Determination of Liraglutide in Rat Plasma Using Selective Liquid Chromatography-Tandem Mass Spectrometry, Mass Spectrometry Letters, 14 (2023), 4.

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