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Sample Preparation Comparison of Large Peptide Quantification in Biological Matrix

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Introduction

In recent years, pharmaceutical research and development has shifted their focus to biologic therapeutics. Liquid chromatography mass spectrometry (LC/MS) has become an alternative method to analyze these large molecules because of its high specificity, sensitivity, and fast method development[1]. At the same time, LC/MS can avoid cross-reactivity and has proven to overcome reagent availability comparing to traditional ligand binding assay (LBA). Here we developed an easy and simple LC/MS method to quantify large peptide drug Exenatide in rat plasma. Our results demonstrate that this easy method can achieve similar quantification limit compared to other vendors who used extensive sample preparation technique.

Experimental

All materials (Acetonitrile, Exenatide, formic acid, 96-well LoBind plates) were purchased from commercial sources.

600 µL of Acetonitrile (1% formic acid) was added to 200 µL of rat plasma aliquots fortified with different concentration of Exenatide, vortex for 5 min and then spin down at 16, 000 g for 10 min. Transfer supernatant to a 96-well plate and then dry down under nitrogen gas with heating. Add 100 µL 20% acetonitrile (0.1% FA) to reconstitute and 20 µL was injected into LC/MS for analysis.

Positive electrospray ionization of Exenatide yielded $[M+5H]^{5+}$ signal at m/z 838.3 as most intense ion, MRM transitions were optimized and 838.3→948.8 was chosen as quantifier and 838.3→396.3 was chosen as qualifier with optimal collision energy.

Figure 1. Agilent Infinity II Bio LC and 6495 triple quadrupole LC/MS system.

Experimental

All data was collected with an Agilent 1290 Infinity II Bio LC coupled to an Agilent 6495 triple quadrupole LC/MS (G6495C) system using the following conditions:

Table 1. UHPLC gradient and conditions

LC Conditions	
Column	AdvanceBio Peptide Mapping 120Å, 2.1 x 150 mm, 2.7 µm (P.N. 653750-902)
Column temperature	60 ºC
Injection volume	20 µL
Autosampler temp	4 ºC
Needle wash	5 seconds in wash port (50:50/water:methanol)
Mobile phase	A = Water + 0.1% formic acid B = Acetonitrile + 0.1% formic acid
Flow rate	0.5 mL/min
Gradient program	Time %B 0 10 8 65 8.2 95 9.2 95 9.3 10 12 10
Stop time	12 min

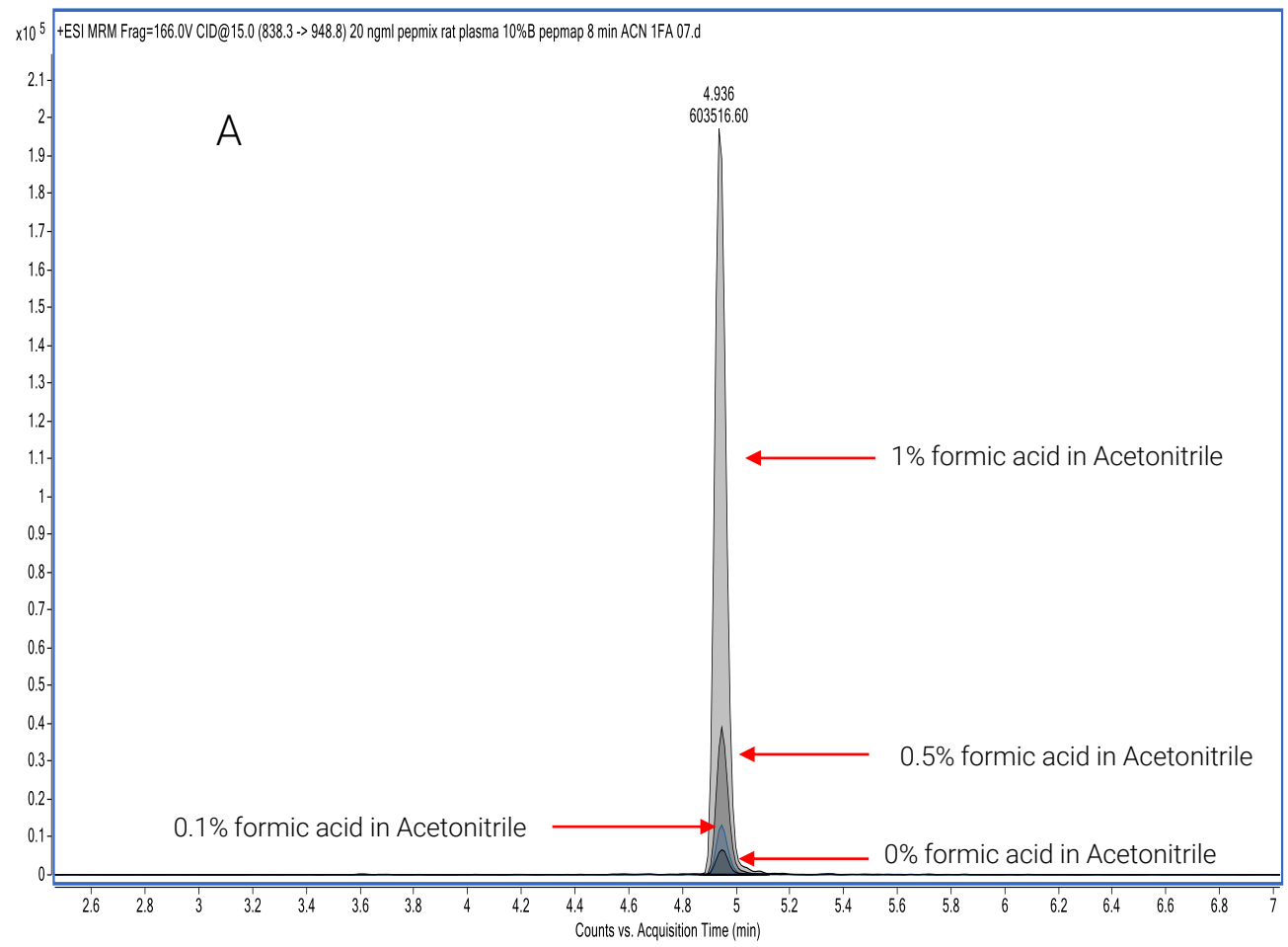
Table 2. 6495 triple quadruple LC/MS conditions.

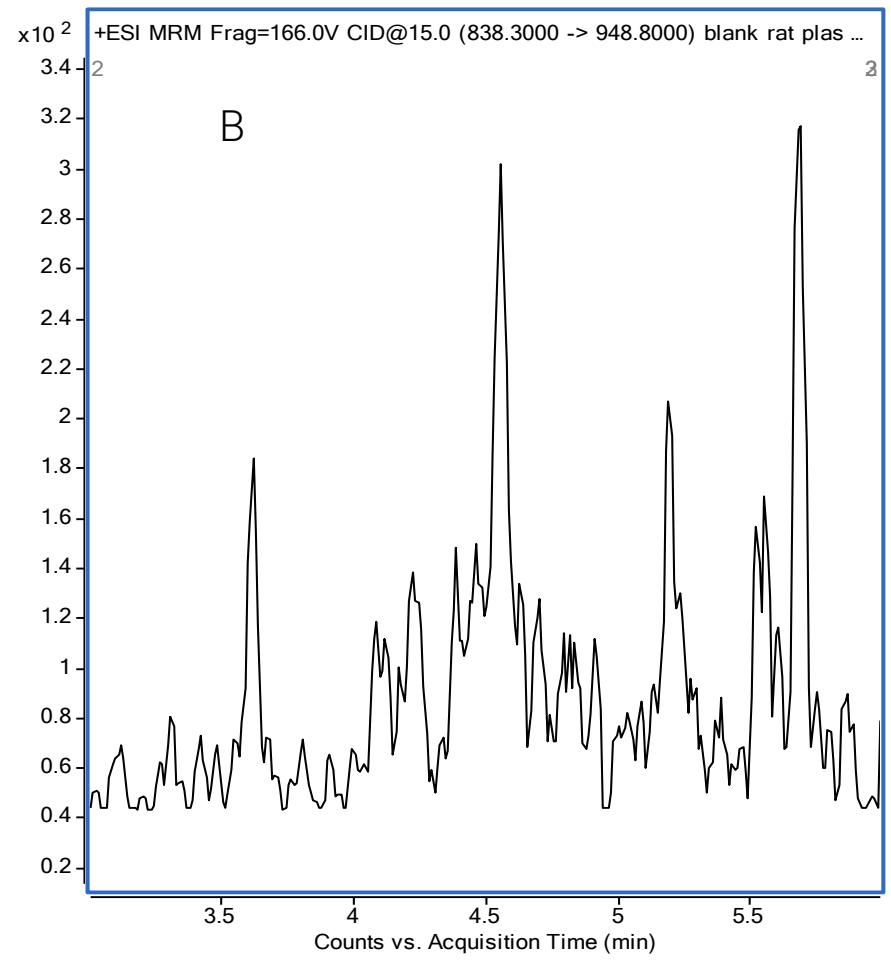
MS Conditions	
Gas temperature	290 ºC
Drying gas flow	18 L/min
Nebulizer gas	35 psi
Sheath gas temperature	250 ºC
Sheath gas flow	12 L/min
Capillary voltage	4500V
Nozzle voltage	1000V
High pressure RF	150 V
Low pressure RF	80 V

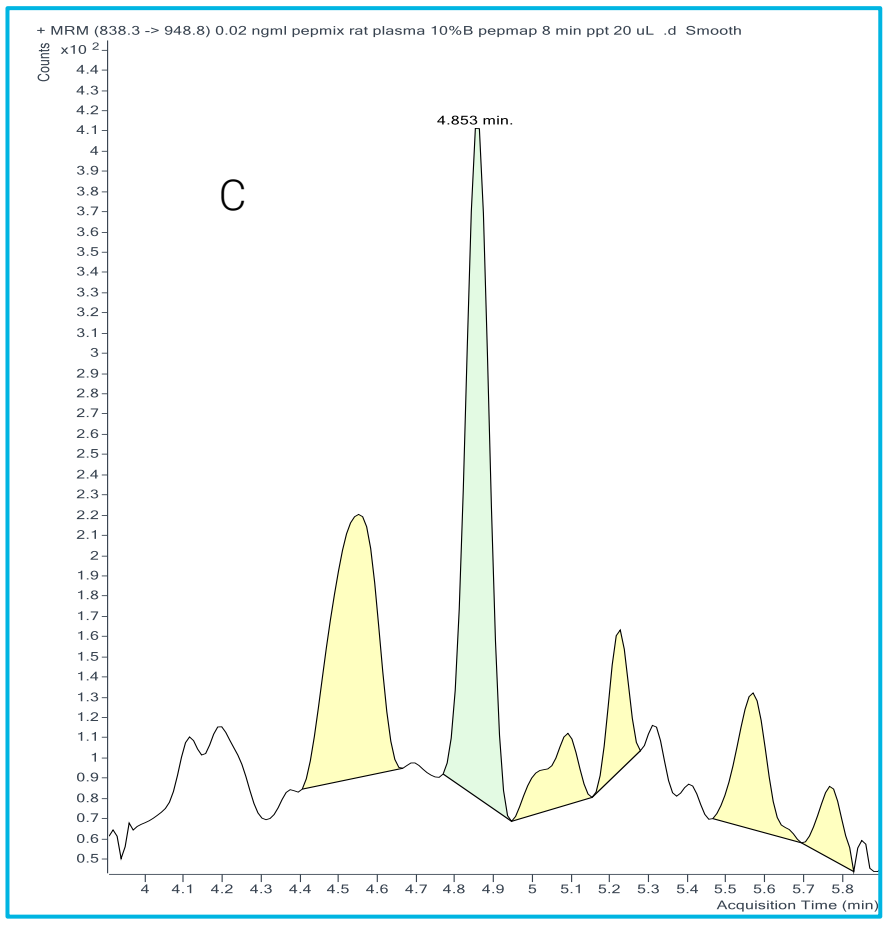
Exenatide LC/MS analysis in rat plasma.

Acetonitrile with 0%, 0.1%, 0.5% and 1% formic acid were evaluated to precipitate proteins from rat plasma, data were shown in Figure 2 (A). Extracted ion chromatogram (EIC) of MRM transition showed 1% formic acid in acetonitrile produced best analyte response, therefore 1% formic acid in acetonitrile was later used for all sample preparation.

MassHunter Quant 10.0 was used to perform quantitative analysis of calibration curve and quality control samples. Blank rat plasma and exenatide low limit of quantification of 0.02 ng/mL as shown in Figure 2 (B) and (C), the calibration curve was linear up to 20 ng/mL with quadratic fit and $1/x^2$ weight as shown in Figure 2 (D), and Table 3.







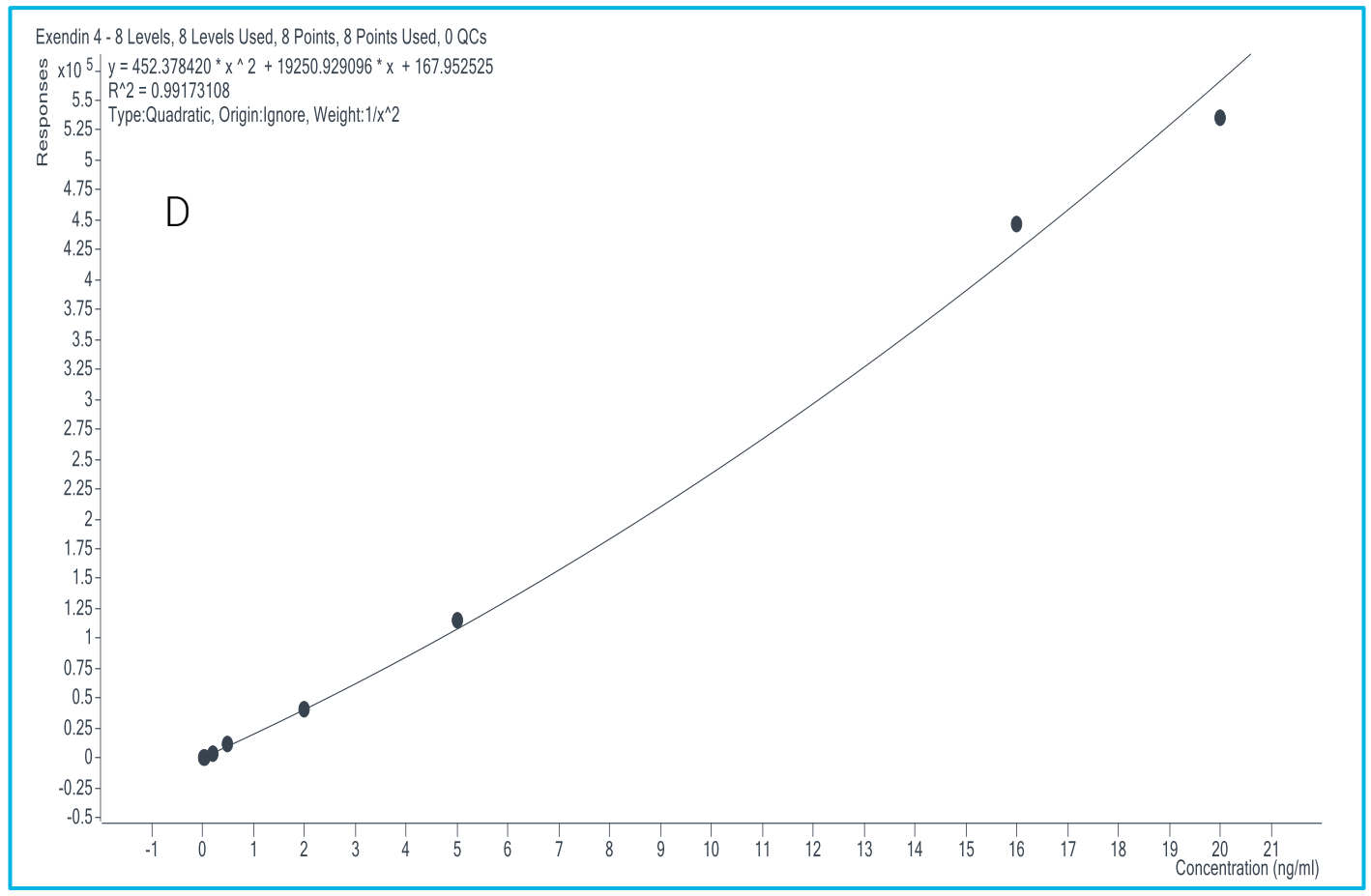


Figure 2. (A) Effects of different concentration of formic acid in acetonitrile. (B) EIC of blank rat plasma. (C) EIC of exenatide lowest calibration point. (D) Calibration curve of exenatide in rat plasma from 0.02 to 20 ng/mL.

Results and Discussion

The intra-day and inter-day analytical precision and accuracy of quality control samples were determined from three independent runs performed over three days. The precision and accuracy results for exenatide in rat plasma were shown in Table 4. All levels of quality control samples (n=6) met acceptance criteria of 20% as recommended by regulatory agency. The results demonstrated excellent assay performance by using simple and easy sample preparation for exenatide quantification.

Table 3. Exenatide calibration curve performance over 3 runs.

Calibration (ng/mL)	0.020	0.040	0.200	0.500	2.000	5.000	16.000	20.000
Mean	0.021	0.044	0.201	0.538	1.978	5.315	15.922	19.855
% Bias	5.25	9.00	0.30	7.62	-1.11	6.31	-0.49	-0.72
% CV	3.70	15.57	12.48	5.55	1.37	0.12	6.63	4.90

Table 4. Exenatide quality control samples precision and accuracy over 3 runs.

	QC concentration (ng/mL)	0.02 (LLOQ)	0.06 (Low)	1.00 (Mid)	15.0 (High)
Run 1	Mean	0.0181	0.0649	1.19	15.3
	% Bias	-9.5	8.2	19.0	2.1
	% CV	12.8	12.0	6.7	3.5
Run 2	Mean	0.0225	0.0593	1.04	15.2
	% Bias	12.3	-1.1	3.7	1.6
	% CV	17.0	15.2	4.5	9.8
Run 3	Mean	0.0218	0.0623	1.10	14.8
	% Bias	9.0	3.8	10.0	-1.3
	% CV	14.6	13.7	8.3	8.6
Inter-day	Mean	0.021	0.0622	1.11	15.1
	% Bias	3.9	3.6	10.9	0.8
	% CV	11.8	4.7	7.7	1.8

Conclusions

- A simple, fast and analytical LCMS method was developed for exenatide peptide quantification in rat plasma. The lower limit of quantification is 20 pg/mL from 200 µL of rat plasma, which is equivalent to other solid phase extraction sample preparation.
- In three qualification runs, intra-day and inter-day QC sample's precision and accuracy all met regulatory acceptance criteria demonstrating excellent assay performance and reproductivity.

References

[1] Jenkins R, et al. Recommendations for validation of LC–MS/MS bioanalytical methods for protein biotherapeutics. AAPS J. 17(1), 1–16 (2015).

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