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アプリケーションノート

A Sensitive and Robust Method for the Quantification of Goserelin in Plasma Using Micro-Elution Plates

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Abstract

In today’ s world of regulated and non-regulated bioanalysis, one of the biggest challenges is addressing upcoming analytical demands, such as the ability to perform LC-MS analysis of large molecules (peptide, protein, oligonucleotides, etc.) with accuracy, reproducibility, and desired sensitivity. This application note demonstrates the benefits of Waters Regulated Bioanalysis System Solution for quantification of large molecules, such as peptides. Oasis WCX Ion-Exchange Micro-Elution Plates, the ACQUITY UPLC System, and the Xevo TQ-S Mass Spectrometer were used in this study for the development of an LC-MS method to analyze pg/mL concentration of goserelin in plasma.

Benefits

Achieves the desired sensitivity and robustness, while retaining the necessary high throughput value and accuracy.

Introduction

Goserelin is a synthetic hormone analogue.[1] Structurally it is a decapeptide, as shown in Figure 1, which is used to suppress the production of the sex hormones (testosterone and estrogen). Goserelin is particularly used in the treatment of breast and prostate cancer. For identification and quantification of goserelin in plasma, a method has been reported in rabbit plasma[2] with an LLOQ of 100 pg/mL with an overall runtime of 10 min. Other methods include radio-immunoassay,[3] capillary zone electrophoresis (CZE)-UV/MS,[4] multiple-injection CZE (MICZE),[5] CE hydrogen deuterium exchange-MS (CE-H/D-MS),[6] and fast atom bombardment-MS (FAB-MS)[7] for crude peptide mixtures only. However, none of these methods address the challenge of analyzing and quantifying goserelin at LLOQ concentrations. In this application note, we report an LC-MS method to determine and quantify goserelin at an LLOQ of 2.5 pg/mL with an overall runtime of 3.5 min in plasma. These results demonstrate the capability of Waters ACQUITY UPLC, Xevo TQ-S, sample preparation and column chemistries to address several bioanalytical challenges, such as achieving the desired sensitivity, addressing upcoming analytical challenges, and regulatory concerns while maintaining high throughput and desired robustness.

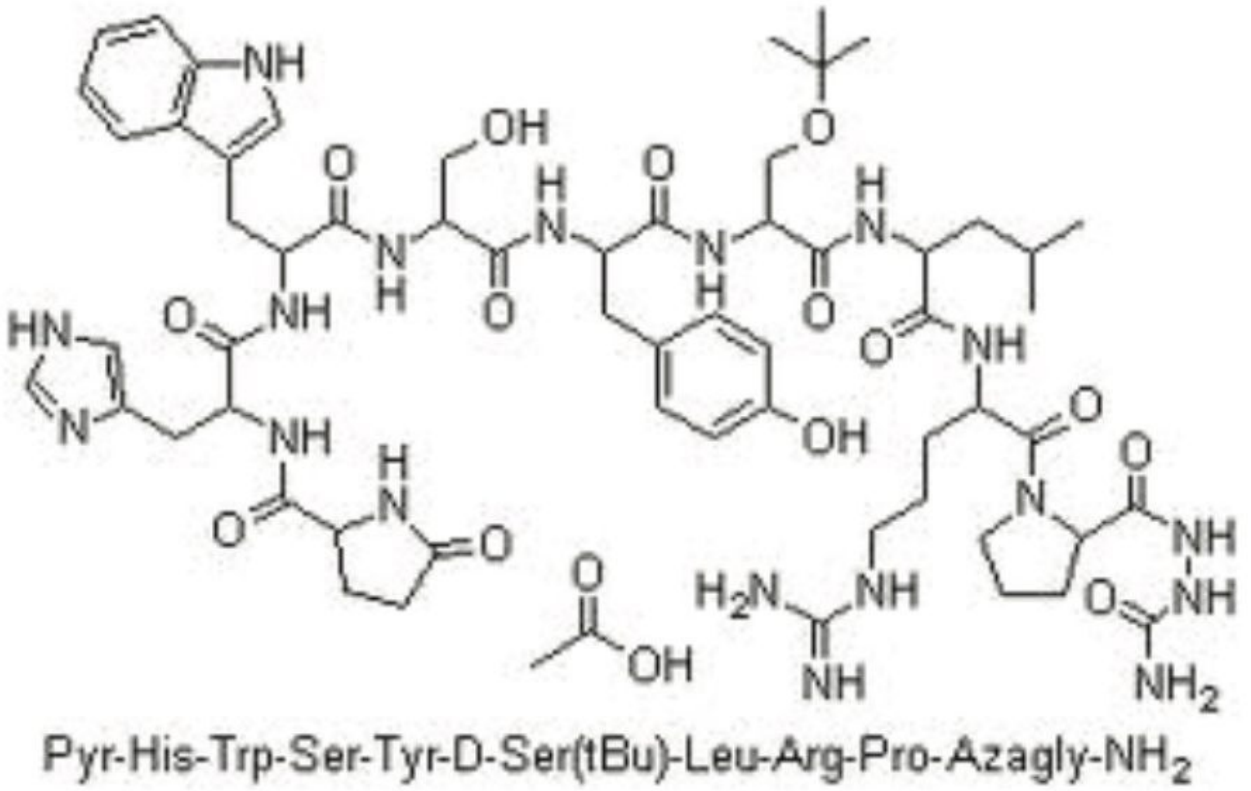


Figure 1. Molecular structure of goserelin and its sequence.

Experimental

Samples were extracted using solid phase extraction (SPE) employing Oasis WCX Micro-Elution 96-Well Plate. An aliquot of plasma was diluted with acidic water, and loaded onto the plate previously conditioned with organic solvent and water. The plasma solution was then washed with water in basic conditions followed by an organic wash, and then eluted using acidified organic elution solvent. The eluted samples were then mixed with Milli-Q water, and injected on to the system. Triptorelin, a decapeptide (M.W. 1311.5), which is a gonadotropin-releasing hormone agonist, was used as an internal standard (IS).

Results and Discussion

Goserelin and triptorelin (IS) eluted at 1.43 min with a peak width of about 5 s, as shown in Figure 2. The

data shown below illustrates the blank signal, shown in Figure 3. The signal obtained from the lower limit of quantification (LLOQ) of goserelin in human plasma is also, shown in Figure 3.

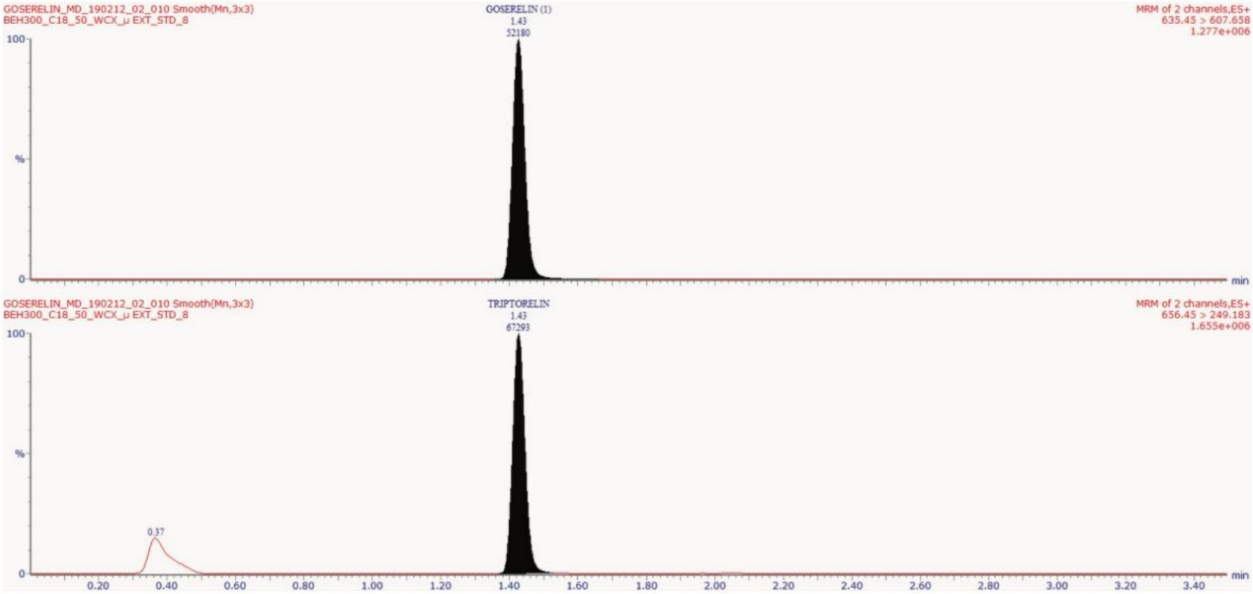


Figure 2. Elution pattern of goserelin and triptorelin.

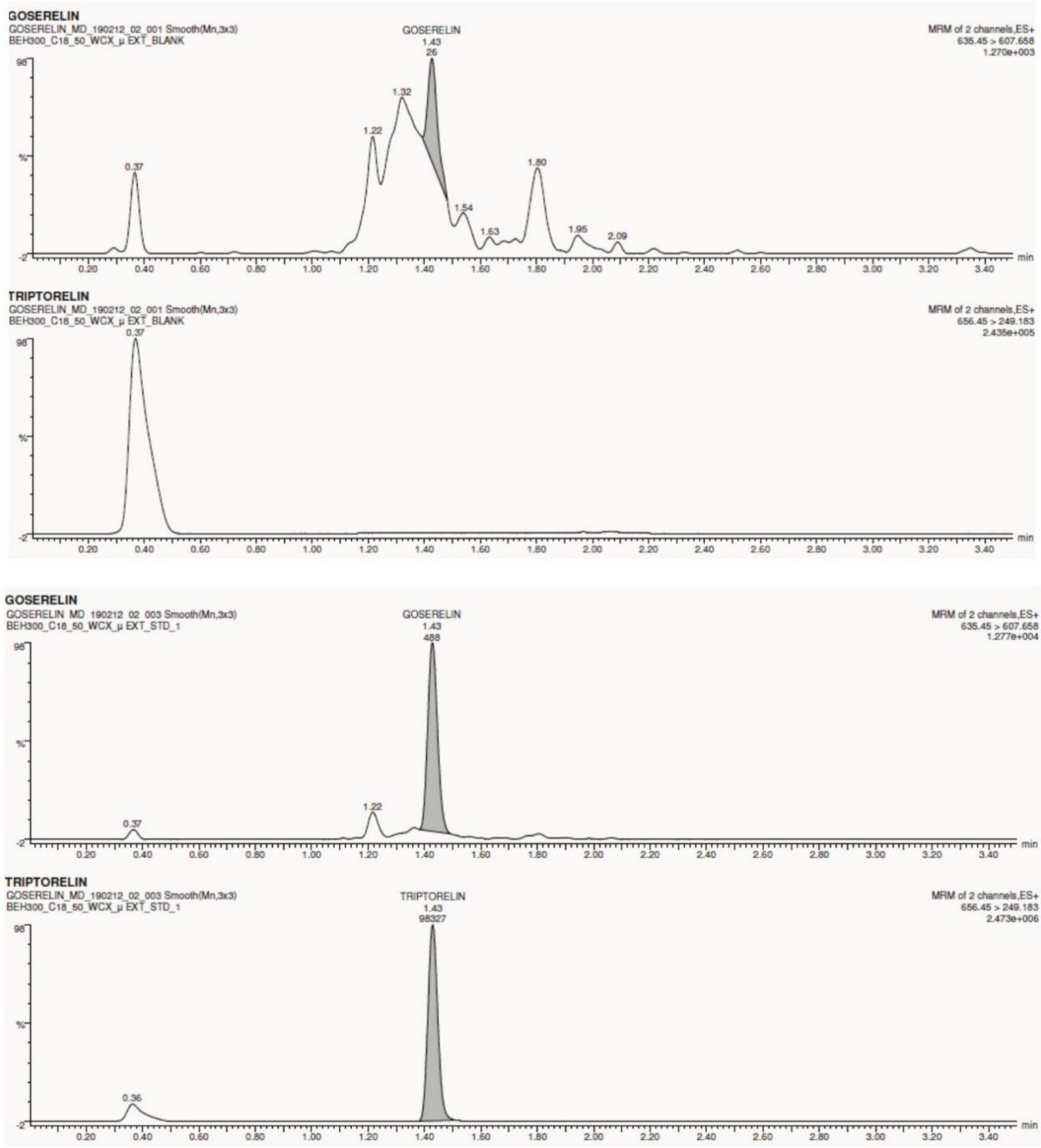
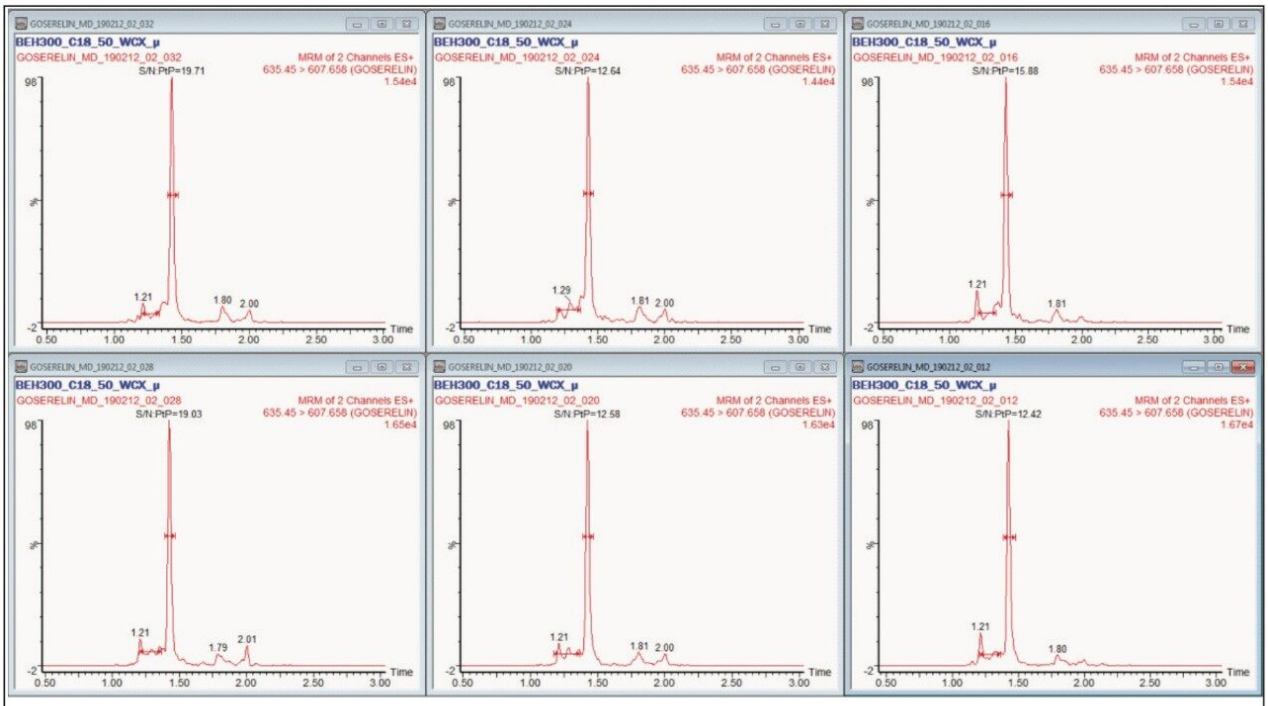


Figure 3. Chromatogram of blank and goserelin at the LLOQ concentration of 2.5 pg/mL.

No significant interference(s) were observed in the retention time (1.43 min) where goserelin elutes, shown in Figure 3. In addition, the signal-to-noise (S/N) ratio of ~16:1 was observed at the LLOQ concentration of 2.5 pg/mL for an average of six replicates of LLOQ samples, as shown in Figure 4.



Signal-to-Noise Ratio of Six Replicates of LLOQ						
LLOQ1	LLOQ2	LLOQ3	LLOQ4	LLOQ5	LLOQ6	AVERAGE
19.71	12.64	15.88	19.03	12.58	12.42	15.38

Figure 4. Chromatogram of LLOQ concentration (2.5 pg/mL) of goserelin with a signal-to-noise ratio of about 16 obtained using ACQUITY UPLC and Xevo TQ-S.

The assay in this report showed linear calibration over the range of 2.5 to 320.0 pg/mL with an excellent r2 value of 0.99, shown in Table 1 and Figure 5. This assay was performed with a 3.5 min injection-to-injection time scale highlighting the capability of ACQUITY UPLC in delivering fast gradients with desired sensitivity, high throughput value, and precision.

Sample	Type	Nominal Conc.	Area	IS Area	Area ratio	Calculated Conc.	Accuracy
EXT_BLK	BLANK		26	4	6.7080	3131.97	
EXT_BLK_IS	ZERO		17	100773	0.0002	0.29	
EXT_CC_1	STD-1	2.5	488	98327	0.0050	2.53	101.2
EXT_CC_2	STD-2	5.0	1032	95435	0.0108	5.26	105.2
EXT_CC_3	STD-3	10.0	1560	85355	0.0183	8.74	87.4
EXT_CC_4	STD-4	20.0	3428	82765	0.0414	19.54	97.7
EXT_CC_5	STD-5	40.0	6866	87965	0.0780	36.65	91.6
EXT_CC_6	STD-6	80.0	13368	77597	0.1723	80.64	100.8
EXT_CC_7	STD-7	160.0	24608	68792	0.3577	167.22	104.5
EXT_CC_8	STD-8	320.0	51096	66860	0.7642	357.00	111.6

Table.1. Calibration data of goserelin over the range of 2.5 to 320.0 pg/mL.

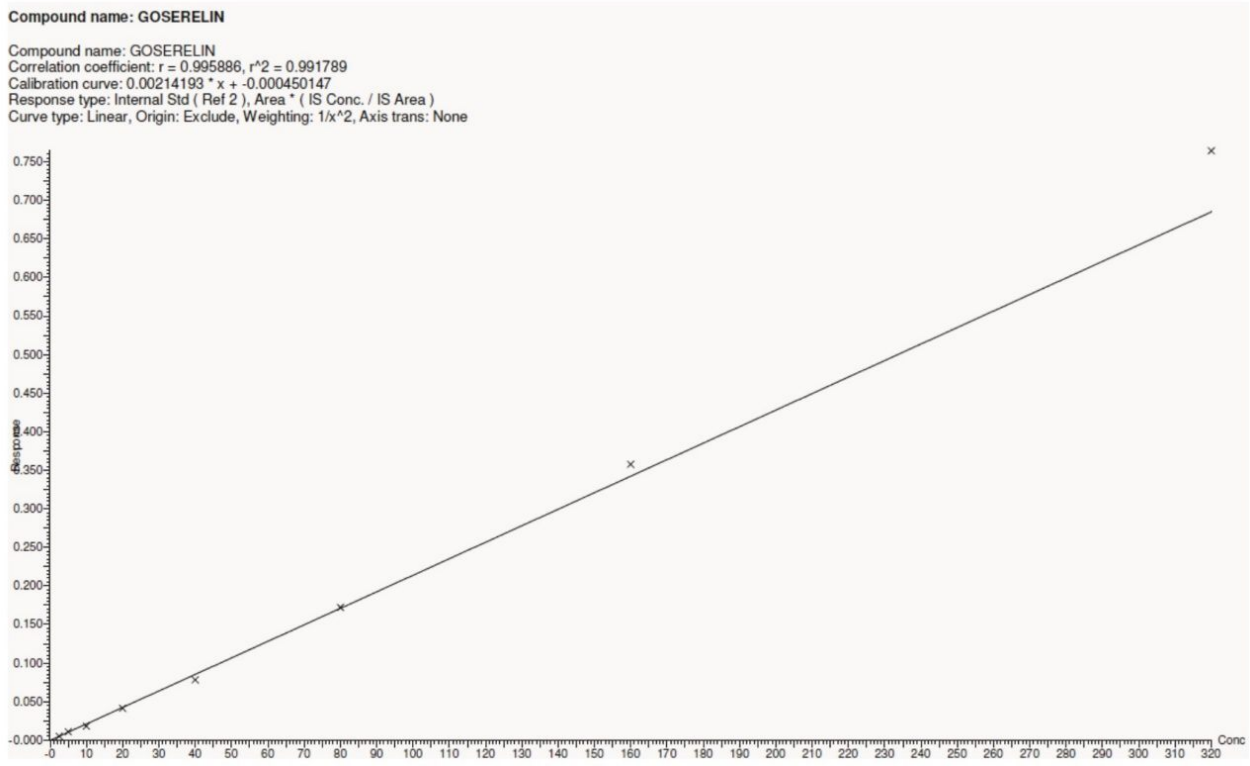


Figure 5. Calibration curve of goserelin.

Recovery of the analyte and internal standard (IS) was calculated by comparing the extracted QC samples against six post-extracted samples and was found to be approximately 66% for the analyte, shown in Tables

2 and 3.

	LQC		MQC		HQC	
S.No	Extracted	Post Spiked	Extracted	Post Spiked	Extracted	Post Spiked
1	1614	2320	14692	20194	24151	41005
2	1706	2479	14493	20253	29566	39894
3	1200	2275	13655	20279	26617	40463
4	1295	2519	14091	20419	27363	42486
5	1562	2448	15043	20797	27176	40887
6	1634	2509	14328	21014	27844	40560
Mean	1502	2425	14384	20493	27120	40883
%Recovery	61.9		70.2		66.3	

Table 2. Analyte recoveries (area under the curve) from six samples of goserelin at LQC, MQC, and HQC concentrations.

LQC	MQC	HQC
61.9	70.2	66.3
Mean analyte recovery (%) = 66.2		

Table 3. Mean analyte recovery (%) of goserelin at LQC, MQC, and HQC levels.

For a comparison of samples within the global batches, four separate batches were analyzed with six samples in each batch at the LLOQQC, LQC, MQC, and HQC concentration levels. The data showed excellent agreement between the six samples in all the four batches, as shown in Table 4. The mean accuracy obtained for all the sample levels was found to be >97% for every concentration, as shown in Table 4. The %CV for repeat batches was found to be within 8% of LLOQQC and varied between 3% and 6% for all QC levels. This variability is well within the acceptable limits in the regulated bioanalysis world.

	LLOQQC	LQC	MQC	HQC
Nominal Conc. (pg/mL)	2.50	10.00	80.00	160.00
P&A-1	2.65	8.84	78.61	158.75
	2.46	9.29	78.31	166.63
	2.65	8.77	78.46	161.05
	2.48	8.95	80.16	168.64
	2.65	9.07	77.98	162.95
	2.59	9.33	78.21	172.10
P&A-2	2.84	10.40	78.24	167.24
	2.82	9.81	77.82	168.72
	2.84	9.74	78.43	168.79
	2.96	9.52	79.76	168.75
	2.72	9.66	78.50	169.23
	2.89	10.09	77.96	167.95
P&A-3	2.67	10.31	77.70	175.98
	2.60	9.93	85.20	164.26
	2.65	10.50	76.41	173.98
	2.79	9.64	83.39	163.49
	2.58	10.47	77.90	173.95
	2.96	9.76	85.75	165.24
P&A-4	2.50	9.79	81.48	151.65
	2.24	9.45	80.66	148.97
	2.50	10.34	78.63	177.09
	2.42	10.50	77.79	155.26
	2.32	9.95	80.72	160.85
	2.33	9.84	85.95	133.38
Mean	2.630	9.748	79.751	164.371
SD	0.1999	0.5263	2.7127	9.7066
%CV	7.6	5.4	3.4	5.9
% Nominal	105.2	97.5	99.7	102.7

Table 4. Comparison of the four separate batches, each containing six goserelin samples at the LLOQQC, LQC, MQ

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