康维讯生物 KANWHISH

官方微信公众号

康维讯生物官网 www.kanwhish.com

Establishment of an Electrochemiluminescent Immunoassay for Quantitative Determination of Semaglutide in Human Serum

Yifan Luo[2], Hefeng Zhang[1,2], Yu Liang[1,2], Linglong Zou[1,2 *]

([1] Suzhou Kanwhish Biotech Co., Ltd., [2] Wenzhou Institute of University of Chinese Academy of Sciences, * Corresponding author)

Introduction

Obesity is a common and easily relapsed chronic metabolic disease. Semaglutide, as one of the representative drugs of GLP-1 receptor agonists, has been approved for the treatment of type II diabetes and obesity. In this study, an immunoassay is developed and validated for the quantitative determination of semaglutide based on CRIVVIN® electrochemiluminescence platform with self-developed monoclonal antibody reagents.

Methods

CRIVVIN® Standard Plate is coated with a mouse anti-semaglutide monoclonal antibody as the capture reagent. The sample is then added to the plate, followed by incubation with biotin-labeled alternative mouse anti-semaglutide monoclonal antibody as the detection reagent to form a sandwich complex (Figure 1). After washing, the plate is incubated with pre-labeled SA-Ru and read upon adding CRIVVIN® Read Buffer. The measured ECL value is proportional to the concentration of the analyte.

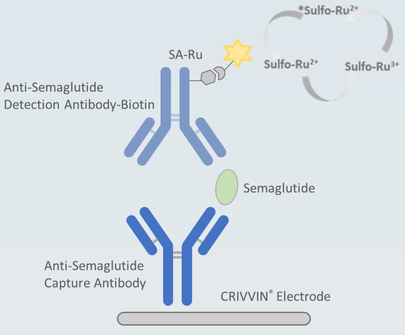

Figure 1. Assay design

Assay Results

Dynamic Range

As illustrated in Figure 2, the quantification range is from 2.0 ng/mL to 240.0 ng/mL and 1,200.0 ng/mL is anchor point.

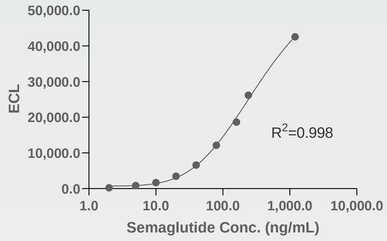

Figure 2. Representative calibration curve

Table 1. Validation results of calibration curve

STD (ng/mL)	MeanConc. (ng/mL)	%CV	%RE	%Total Error
240.0	249.6	7.4	4.0	11.4
160.0	150.7	6.1	-5.8	11.9
80.0	80.2	3.3	0.3	3.6
40.0	40.8	3.6	2.1	5.7
20.0	20.4	3.4	1.8	5.2
10.0	10.3	4.6	3.3	7.9
5.0	4.8	5.1	-3.6	8.7
2.0	2.0	1.8	1.2	3.0

Accuracy and Precision (A&P)

The assay was performed in 6 A&P runs over 3 days with 5 levels of QC samples to assess precision and accuracy. The results are as summarized in Table 2. Both accuracy (%RE) and precision (%CV) meet the requirements.

Table 2. Validation results of accuracy and precision

QCs	Nominal (ng/mL)	MeanConc. (ng/mL)	%CV	%RE	%Total Error
ULOQ	240.0	241.9	10.0	0.8	10.8
HQC	180.0	167.9	13.9	-6.7	20.6
MQC	80.0	79.4	10.3	-0.8	11.1
LQC	6.0	5.7	7.3	-5.3	12.6
LLQC	2.0	2.0	6.8	-0.6	7.4

Dilution Linearity and Hook Effect

Dilution linearity was assessed with QC samples serially diluted in human serum. Hook effect was assessed with a QC sample prepared at 2,400.0 ng/mL, 10 times above the ULOQ. Dilution linearity was demonstrated up to 200-fold dilution, without hook effect at 2,400.0 ng/mL (Table 3).

Table 3. Validation results of dilution linearity and hook effect

Dilution Factor	Nominal (ng/mL)	MeanConc. (ng/mL)	%CV	%RE	Results
1	2,400.0	2,177.7	2.8	-9.3	No Hook effect
10	240.0	220.7	14.3	-8.0	Pass
50	48.0	53.3	9.6	11.1	Pass
100	24.0	24.0	7.1	0.1	Pass
200	12.0	11.8	5.5	-1.8	Pass

Selectivity

Selectivity was evaluated with 10 individual human serum samples, and the results are summarized in Table 4, which indicates that there is no matrix effect.

Table 4. Validation results of Selectivity

Sample ID	BLK			LLOQ (2.0 ng/mL)			ULOQ (240.0 ng/mL)		
	Conc. (ng/mL)	%CV	%RE	Conc. (ng/mL)	%CV	%RE	Conc. (ng/mL)	%CV	%RE
S1	BQL	1.6	N/A	2.2	0.0	9.6	236.6	6.1	-1.4
S2	BQL	0.0	N/A	2.4	1.1	19.0	273.6	3.6	14.0
S3	BQL	2.3	N/A	2.4	1.9	20.4	221.3	2.5	-7.8
S4	BQL	5.1	N/A	2.0	6.0	1.0	214.6	3.3	-10.6
S5	BQL	0.4	N/A	1.7	2.3	-12.9	193.2	3.9	-19.5
S6	BQL	1.2	N/A	2.2	3.5	7.9	226.2	3.6	-5.8
S7	BQL	2.8	N/A	2.1	0.2	4.0	208.1	14.8	-13.3
S8	BQL	0.5	N/A	2.0	5.5	-0.2	248.7	5.1	3.6
S9	BQL	2.4	N/A	2.0	4.4	-2.1	240.0	11.5	0.0
S10	BQL	1.1	N/A	2.2	9.7	8.6	259.3	5.2	8.1

Specificity

Semaglutide has 94% structural homology to native human GLP-1. To assess assay specificity, serum samples were spiked with various levels of GLP-1 to mimic the samples with potential endogenous interference. As shown in Table 5, the assay is specific to semaglutide.

Table 5. Validation results of specificity

GLP-1 Conc. (ng/mL)	Nominal (ng/mL)	MeanConc. (ng/mL)	%CV	%RE
200.0	80.0	71.8	2.4	-10.2
100.0		79.2	6.7	-1.0
50.0		73.0	5.3	-8.8
25.0		71.1	9.5	-11.1

Stability

To evaluate sample stability, QC samples were stored under the following conditions: up to 24 hours at room temperature (RT), 3 days at 2 ~ 8℃, 7 days at 2 ~ 8℃, and 5 freeze-thaw cycles (F/T). If the experimental results of %CV and %RE are both within ±20%, the stability testing passes the acceptance criteria. As shown in Table 6, the test results demonstrate sample stability at all conditions.

Table 6. Validation results of stability

Condition	Sample ID	Nominal (ng/mL)	MeanConc. (ng/mL)	%CV	%RE
24 hours at RT	HQC	180.0	178.2	2.6	-1.0
	LQC	6.0	5.9	0.4	-2.6
3 days at 2 ~ 8℃	HQC	180.0	182.0	8.4	1.1
	LQC	6.0	5.6	5.1	-6.0
7 days at 2 ~ 8℃	HQC	180.0	172.8	4.8	-4.0
	LQC	6.0	5.6	0.9	-6.2
5 F/T	HQC	180.0	177.6	8.2	-1.4
	LQC	6.0	5.8	8.3	-3.8

Conclusion

This method is suitable for the determination of semaglutide in human serum and can support pharmacokinetic bioanalysis in clinical studies. The quantitative range of the method is from 2.0 to 240.0 ng/mL. Method development confirmed sufficient accuracy, precision, dilution linearity, selectivity, specificity, and sample stability. The study also shows that CRIVVIN® ECL platform is a reliable for bioanalysis.

© 2024 CBF China