Electronic Supplementary Information (ESI)

An ultrasensitive photoelectrochemical bioanalysis strategy for tumor maker based on the significantly enhanced signal of bismuth oxyiodine microspheres/graphitic carbon nitride composites

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Experimental section

Materials and reagents.

Bismuth nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$), 1-ethyl-3-[3-(dimethylamino-propyl)] carbodiimide (EDC), n-hydroxysuccinimide (NHS) was gained from Sigma-Aldrich company. Polyethylenimine (PEI) was purchased from Aladdin company. Glutaraldehyde (25% aqueous solution), tris (hydroxymethyl) aminomethane (Tris) and 3-mercaptopropionic acid (MPA) was bought from Alfa Aesar company. Sodium hydroxide (NaOH) and copper (II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) were purchased from Shanghai Chemical Reagent company. Glycol was obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd., and hydrogen peroxide (H_2O_2) and potassium iodide (KI) was purchased from Sinopharm Chemical Reagent Co. Ltd., Capture CEA antibody (Ab_1) and signal CEA antibody (Ab_2) were acquired from

Boson Biotech Co. Lth. Carcinoembryonic antigen (CEA, Ag), prostate-specific antigen (PSA), α-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9) were obtained from Shanghai Linc-Bio Science Co. Ltd. (China). Clinical serum samples were provided by Jiangsu Institute of Cancer Prevention and Cure (China), and they were obtained by centrifugation of blood for about 5 min with the rotation rate of 3000-4000 rpm (The Helsinki number was not needed in China.).

All of the other reagents were of analytical reagent grade. Ultrapure water was used in all the process from Millipore water purification system (≥18 MΩ.cm, Millipore SAS Corporation, France). The washing solution was Tris-HCl buffered saline (pH 7.4, 10 mmol·L^{-1}). The supporting electrolyte solution was Tris-HCl buffered saline (pH 7.4, 0.1 mol·L^{-1}). Indium tin oxide (ITO) glasses supplied by Jintan Kondrk Photoelectric Science＆Technology Co. Itd (China) were used as the work electrodes.

Apparatus.

Transmission electron microscopy (TEM, H-7650, JEOL, Japan) and scanning electron microscopy (SEM, JSM-7600F, JEOL, Japan) were used to characterize the materials. The UV-visible (UV-vis) absorption spectra were tested on a Cary 60 spectrophotometer (Agilent, USA). The photoluminescence (PL) spectra were collected on a Fluoromax-4 spectrofluorometer (Horiba, USA) at an excitation wavelength of 360 nm. The slit width was 5.0 nm. Electrochemical impedance spectroscopy (EIS) were determined on an Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, Netherland) in KCI solution (0.1 mol·L^{-1}) containing a $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5.0 mmol·L^{-1}) (1:1) mixture as a redox probe from 0.1 Hz to 100 KHz with a signal amplitude of 10 mV. In addition, the photocurrent was measured on a PEC workstation (Zahner, Germany) with a three-electrode system: a modified electrode with a geometrical area of 0.09π cm^2 as the working electrode, a Pt

wire as the counter electrode, and a saturated Ag/AgCl electrode as the reference electrode.

Preparation of BiOI/g-C_3N_4 composites suspension.

BiOI microspheres were synthesized through the previous work with some modification.[S1] Briefly, 10 mL of 0.10 mol·L^{-1} $Bi(NO_3)_3·5H_2O$ dissolved in glycol was added dropwise to 10 mL of 0.10 mol·L^{-1} KI dissolved in glycol. After vigorously stirring at room temperature for 1h, the mixture was transferred into a 50 mL Teflon-lined stainless steel autoclave, which was heated to 160 °C and maintained for 12 h. Until cooling, the product was washed three times with ultrapure water and ethanol, and then it was dried in vacuum oven. The g-C_3N_4 was prepared via a reported method with slight alteration.[S2] 15.0 g of urea was added to 20.0 mL of ultrapure water in an alumina crucible, which was placed in a muffle furnace. Subsequently, the temperature increased to 400 °C and maintained for 1.0 h. Finally, the temperature rose to 450 °C and held for 5.0 h to obtain g-C_3N_4. The g-C_3N_4 was not further pretreated by acidification or oxidation. By directly mixing BiOI (2 mg·mL^{-1}) and g-C_3N_4 (1.2 mg·mL^{-1}) suspensions at the same volume and then being sonicated for 30 min, the BiOI/g-C_3N_4 composites suspension was prepared.

Synthesis of Ab_2-CuS conjugates.

CuS NPs was synthesized according to the previous research with some modification.[S3] 6 μL MPA was added into the solution of $Cu(NO_3)_2·3H_2O$ (20 mL, 2 mmol·L^{-1}) with vigorous stirring. After the pH of the solution was regulated to 9.0 with NaOH aqueous solution, the mixture was bubbled with highly pure N_2 for 30 min. Then, 20 mL of 5 mmol·L^{-1} Na_2S solution was added dropwise to the mixture and stirred for 24 h under N_2 atmosphere to gain CuS NPs suspension. Ab_2-CuS

conjugates were prepared referring to the reported method with some modification.[S3] 100 μL of aqueous solution containing 20 mg·mL^{-1} EDC and 10 mg·mL^{-1} NHS was added into 1mL of CuS Nps suspension for 1h to activate the carboxylic groups. After centrifugation and removal of the supernatant solution, 1 mL of Ab_2 (0.1 mg·mL^{-1}) was added to the precipitate and stirred for 12 h at 4 °C. Subsequently, the mixture was centrifuged and washed by the washing solution to obtain the Ab_2-CuS conjugates. The Ab_2-CuS conjugates were dispersed in 1 mL of Tris-HCl buffered saline (pH 7.4, 10 mmol·L^{-1}) at 4 °C in the fridge for future use.

Fabrication of the PEC biosensing platform.

A piece of bulked ITO (sheet resistance 20-25 Ω/square) was incised to small pieces of rectangular ITO. Before modification, the ITO glass was cleaned by successive sonication in acetone, mixed solution of ethanol and 1 mol·L^{-1} NaOH (v/v, 1:1), respectively, and then it was ultrasonically rinsed with ultrapure water for 15 min. After being dried in stove, the nonconductive rubberized fabric with hollow-carved circle of 0.09π cm^2 (the diameter was 0.6 cm) was pasted on the small piece of rectangular ITO to obtain the modified electrode (Fig. S1A). Then 20 μL of the BiOI/g-C_3N_4 composites were dropped on an ITO electrode with 0.09π cm^2 exposed area and dried. Next, 20 μL of PEI solution was applied to the modified ITO electrode and dried in vacuum oven at 60 °C. The obtained PEI/BiOI/g-C_3N_4/ITO electrode was immersed into 20 μL of glutaraldehyde (2.5%) for 30 min and washed with the washing solution. Whereafter, 20 μL of Ab_1 (50 μg·mL^{-1}) was dropped on the above modified electrode, and the obtained modified electrode was incubated at 4 °C overnight. After a gentle wash, 20 μL of BSA (1%) aqueous solution was dropped on the electrode to block nonspecific binding sites for 1 h at 4 °C. Finally, the

BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO was rinsed with the washing solution to obtain the biosensing platform.

Measurement procedure.

The fabricated biosensor was incubated with 20 μL of various concentrations of CEA at 37 °C for 1 h. Finally, the CEA/BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO electrode was washed and incubated with 20 μL of Ab_2-CuS conjugates (10%) at 37 °C for 1 hour. After washing the electrode, Ab_2-CuS/CEA/BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO was obtained (Fig. S1B) and it was inserted into the supporting electrolyte solution with 0.03 mol·L^{-1} H_2O_2 at an applied potential of -0.05 V under 405 nm irradiation to record the PEC response for CEA detection. The different electrodes were used for continuous detection of CEA from low concentration to high value. The PEC detection system did not need deaeration with nitrogen. All the error bars in this work originated from 3 times of parallel measurement.

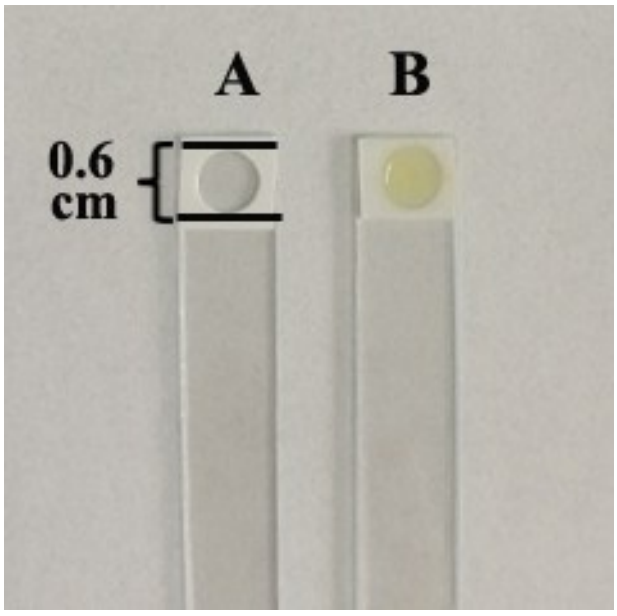


Fig. S1. Photographs of (A) bare ITO electrode and (B) Ab_2-CuS/CEA/BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO electrode.

Characterization

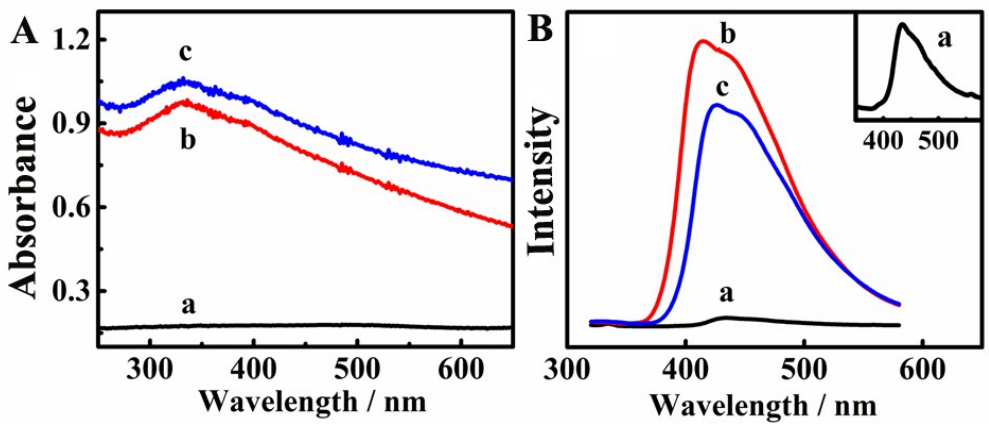


Fig. S2. (A) UV-vis spectra and (B) PL spectra of (a) BiOI microspheres, (b) g-C_3N_4 and (c) BiOI/g-C_3N_4 composites. Inset: amplified PL spectrum of BiOI microspheres.

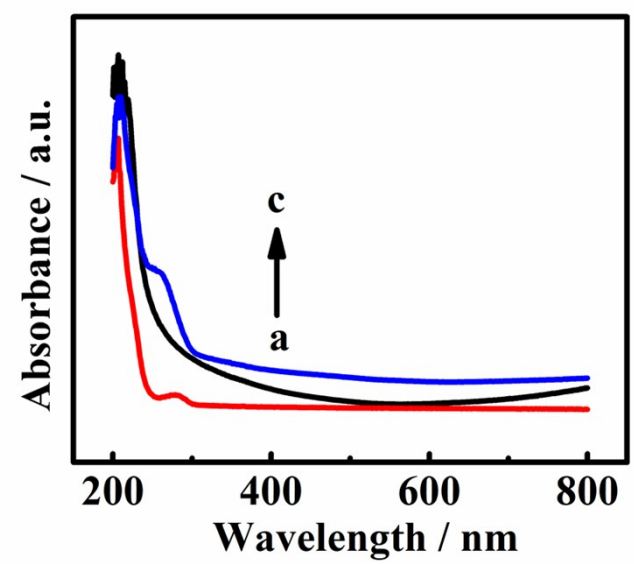


Fig. S3. UV-vis absorption spectra of (a) Ab_2, (b) CuS NPs and (c) Ab_2-CuS conjugates.

The UV-vis spectra were used to confirm the formation of Ab_2-CuS conjugates (Fig. S3). The CuS NPs did not display any evident absorption peak (200-800 nm) (curve b). Nevertheless, the UV-vis spectrum of Ab_2 showed an obvious UV absorbance peak at 280 nm (curve a). When Ab_2 molecules conjugated with CuS NPs,

the absorbance peak exhibited hypochromatic shift (curve c), which might verify the formation of Ab_2-CuS conjugates.

PEC responses

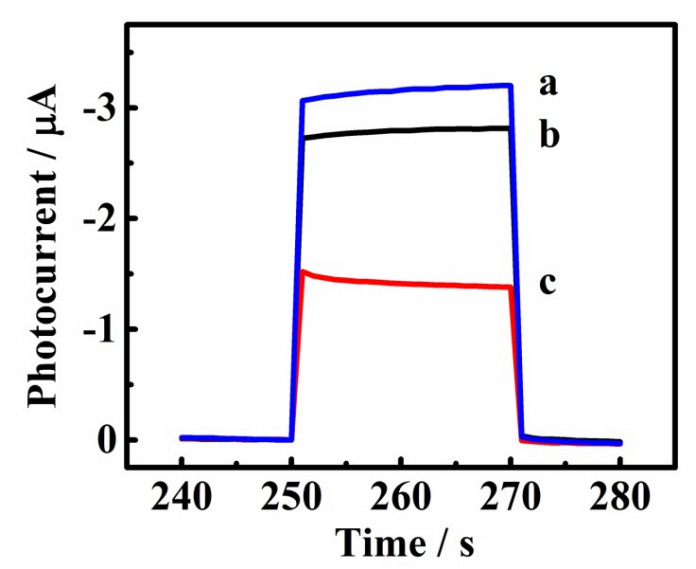


Fig. S4. Photocurrent responses of the different modified ITO electrodes (a) CEA/BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO, (b) Ab_2/CEA/BSA/Ab_1/PEI/BiOI/g-C_3N_4/ITO and (c) Ab_2-CuS/ CEA/BSA/Ab1/PEI/BiOI/g-C_3N_4/ITO electrodes**.**

Optimization of the detection conditions

To obtain the intensive PEC signal for promoting the sensitivity of the designed sandwich bioanalysis, some experiment conditions were optimized in Fig. S5. The irradiation wavelength was significant for the generation of photocurrent. The photocurrent intensity declined when the excitation wavelength increased from 380 to 470 nm (Fig. S5A) due to that BiOI/g-C_3N_4 composites were likely prior to absorbing the light of short wavelength, which was consistent with the result of the UV-vis absorbance spectrum (Fig. S2A, curve c). While the photocurrent was 6.18 μA at 405

nm, which was 2.43 times of the photocurrent (2.54 μA) at 430 nm. The photocurrent at 405 nm could be enough intensive for satisfying the sensitivity of the proposed PEC bioanalysis. In addition, the ultraviolet light of high energy might make the molecules denatured or even inactivated. Considering the two aspects, 405 nm was selected as the irradiation wavelength for PEC bioanalysis.

The bias potential was another key factor to influence the PEC performance. Fig. S5B showed the photocurrent intensity of BiOI/g-C_3N_4 composites modified ITO electrode at different bias potential from 0.05 to -0.15 V, it was evident that the photocurrent reached a maximum value at -0.05 V. Moreover, the low bias potential was beneficial to eliminate the interference and less harmful to the biomolecules. Therefore, -0.05 V was chosen for the PEC measurement.

H_2O_2 acted as an effective electron acceptor for remarkably enhancing PEC response. The photocurrent intensity dramatically promoted with increasing the H_2O_2 concentration from 0 to 0.03 mol·L^{-1}, and then it reached a plateau when the H_2O_2 concentration rose from 0.03 to 0.05 mol·L^{-1} (Fig. S5C), suggesting the equilibrium concentration of H_2O_2 for PEC reaction. Thus, 0.03 mol·L^{-1} was as the optimized concentration for PEC detection.

The PEC performance was depended on the concentration of light-sensitive materials. The concentration of BiOI microspheres suspension was fixed at 2 mg·mL^{-1}. While the concentration of g-C_3N_4 increased from 0.4 to 1.2 mg·mL^{-1}, the photocurrent sharply improved with increasing the concentration of g-C_3N_4 from 0.4 to 1.2 mg·mL^{-1} and it leveled off at the concentration from 1.2 to 2.0 mg·mL^{-1} (Fig. S5D), forecasting the saturated concentration of g-C_3N_4 for PEC reaction. Accordingly, 1.2 mg·mL^{-1} g-C_3N_4 suspension was selected to add into the same volume of 2 mg·mL^{-1} BiOI microspheres suspension for the preparation of BiOI/g-

C_3N_4 composites suspension.

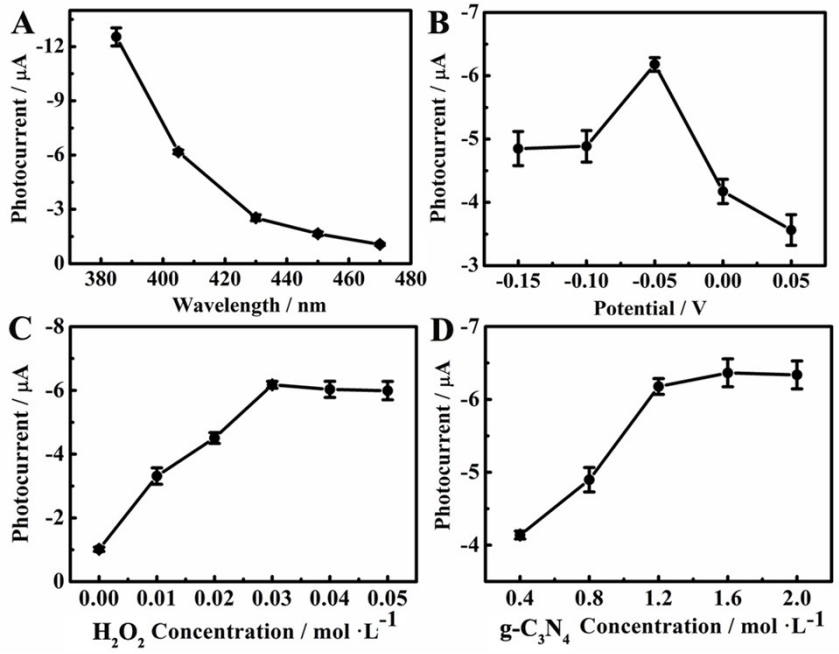
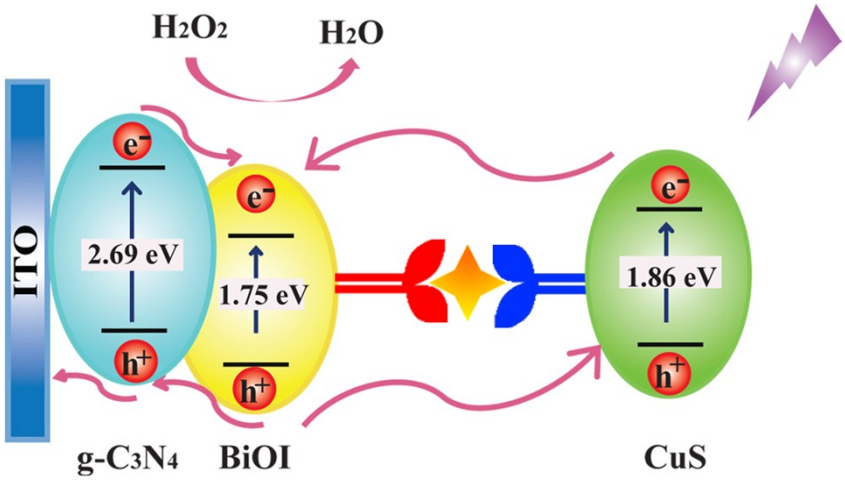


Fig. S5. Effects of (A) the excitation wavelength, (B) the bias potential and (C) the concentration of H_2O_2 on the PEC response of BiOI/g-C_3N_4/ITO electrode. (D) Influence of the concentration of g-C_3N_4 on the PEC signal at the BiOI concentration of 2 mg·mL^{-1}.

PEC bioanalysis

The probable mechanism of the sandwich PEC bioanalysis was discussed (Scheme S1). In the absence of target, the biosensor exhibited a relatively large photocurrent response, because the cascade band-edge levels between g-C_3N_4 and BiOI made BiOI/g-C_3N_4 composites displayed high light absorbance and low electron-hole recombination.[S4, S5] In the presence of target, the Ab_2-CuS conjugates specifically bound with target, generating the decline of the PEC response. On the one hand, the large steric hindrance of the Ab_2-CuS conjugates hindered the transfer of photogenerated electron and electron acceptor (H_2O_2). On the other hand, the CuS

NPs could competitively absorb the exciting light and consume electron acceptor. Meanwhile, the photo-induced holes on BiOI transferred on the opposite direction due to equilibrium of the different Fermi levels,[S6-S8] which impeded the transport of photo-induced holes to the electrode.[S9] Thus, the obvious change of photocurrent before and after introducing with target and Ab_2-CuS conjugates could contribute to the ultrahigh sensitivity of the proposed sandwich bioanalysis.



Scheme S1. Photogenerated electron-hole transfer of the sandwich PEC biosensing.

The PEC responses in the presence of interference alone was investigated (Fig. S6). Compared to the photocurrent response of blank sample, the relative errors of the variation of the photocurrent measured in the presence of single interfering protein intensity was within 2.5%, which suggested a negligible interference. The results further confirmed the excellent selectivity of the developed PEC bioanalysis strategy.

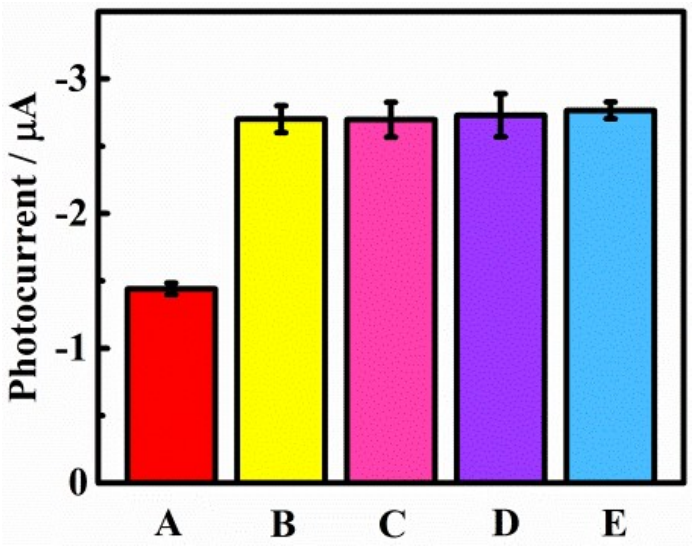


Fig. S6. Photocurrent responses of the proposed PEC biosensor towards (A) 100 pg·mL^{-1} CEA, (B) 100 U·mL^{-1} CA19-9, (C) 100 pg·mL^{-1} PSA, (D) 100 pg·mL^{-1} AFP and (E) blank sample.

Table S1. Comparison of the proposed PEC biosensing strategy with the analytical performances of other methods.

Detection technique	Linear range (ng·mL^{-1})	Detection limit (ng·mL^{-1})	Reference
Electrochemistry	1.0×10^{-4} – 20	2.0×10^{-5}	S10
Electrochemiluminescence	2.0×10^{-2} – 80	6.8×10^{-3}	S11
Fluoremetry	2.0×10^{-3} – 0.10	8.0×10^{-4}	S12
Colorimetry	1.0 – 1.0×10^{4}	0.16	S13
Photoelectrochemistry	1.0×10^{-3} – 90	3.3×10^{-4}	S14
Photoelectrochemistry	1.0×10^{-5} – 10	5.3×10^{-6}	This work

PEC assay of human serum samples

Table S2. Comparison of CEA assays in human serum samples using the developed and reference methods.

Simple Number	a	b	c	d
Developed method ($ng \cdot mL^{-1}$)	39.6	2.2	110.1	45.0
Reference method ($ng \cdot mL^{-1}$)	36.9	2.1	108.2	51.9
Relative error (%)	7.3	4.8	1.8	13.3

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