Electronic Supporting Information

A novel strategy to evaluate the degradation of quantum dots: identification and quantification of CdTe quantum dots and corresponding ionic species by CZE-ICP-MS

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Experimental section

S1. Chemicals

Aqueous dispersions of L-glutathione (L-GSH)/L-cysteine (L-Cys)-capped CdTe QDs used in this study were synthesized by our research group. Potassium borohydride (KBH_4, ≥95%), cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, ≥99.0%), L-GSH (reduced form, BR) and L-Cys (BR) and Te powder (high purity reagent) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Beijing，China). Sodium hydroxide, boric acid, and sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) were obtained from Sigma-Aldrich (USA). Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), SDS, CTAB, Tween 20 and EDTA were bought from Amresco (Ohio, USA). The standard stock solutions of Cd (1 g/L) and TeO_3^{2-} (1 g/L, calculated as Te, the same as below) were prepared by dissolving $CdCl_2$ (99.99%, Aladdin, Shanghai, China) and sodium tellurite (Na_2TeO_3, 99.7%，Aladdin, Shanghai, China) in high purity deionized water, respectively. The standard stock solutions of Indium (In, 1 g/L) containing 1 M HNO_3 was provided by the National Standard Substances Center (Beijing, China). Human serum albumin (HSA) was acquired from Sigma (USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS, Australia source) were purchased from Corning Cellgro (Manassas, VA, USA). Normal rat serum was obtained from Applygen Technologies Inc. (Beijing, China). Water used in the experiments was high purity grade with a conductivity of 18.2 MΩ cm. All other reagents were at least of analytical reagent grade. All chemicals were used without any further purification.

S2. CZE-ICP-MS measurement

CE separation was performed on a 7100 CE system (Agilent Technology, Waldbronn, Germany). Coated fused-silica capillaries (i.d. 75 μm; o.d. 360 μm; length 100 cm) were obtained from Yongnian Reafine Chromatography Co., Ltd. (Hebei, China). A new capillary was initialized by flushing it with 1 M NaOH for 10 min, and keeping the solution in the capillary for 10 min. Then, the capillary was rinsed with water for 10 min, followed by running buffer for 20 min. The capillary was conditioned with the initialization program each day before use. Between each run, the capillary was rinsed sequentially with 0.1 M NaOH and water for 3 min, and then equilibrated

with the running buffer for 5 min. All conditioning and rinsing steps above were performed under a pressure of 970 mbar to eliminate the possibility of a memory effect. Samples were hydrodynamically injected at 35 mbar for 5 s. The temperature of the cartridge was set at 25 °C (ambient temperature), and the applied voltage for the separation was 16 kV. All solutions (samples, different concentrations of NaOH, water and buffer solution) were filtered through a 0.22 μm microfiltration membrane syringe filter before use.

The hyphenation of CE with ICP-MS (8800 ICP-MS Triple Quad, Agilent Technology, Tokyo, Japan) was realized by directly connecting the outlet of the capillary to a CE ESI Sprayer II (Agilent Technology, Germany). An aqueous solution containing 1μg/mL In was used as the make-up solution for monitoring the stability of the CE flow, and it was introduced to the spray chamber as a sheath liquid at 5 μL/min through the CE ESI Sprayer II. An insulated copper wire with a crocodile clip head was used to connect the CE ESI Sprayer II (as the cathode in CE) to grounding device of the ICP-MS.

The ICP-MS was operated under Helium (He) gas mode, and the mass isotopes of ^{111}Cd and ^{125}Te were monitored for CdTe QDs and ^{115}In for the make-up solution. The details of the operation conditions are listed in in Table S1.

Table S1 Operation conditions of ICP-MS

Parameters	**Value**
RF power	1550 W
plasma gas (Ar) flow rate	15.0 L/min
auxiliary gas (Ar) flow rate	0.9 L/min
carrier gas (Ar) flow rate	1.3 L/min
make up gas (Ar) flow rate	1.0 L/min
sampling depth (mm)	8.0 mm
monitored isotopes	^{111}Cd, ^{125}Te, and ^{115}In
peristaltic pump speed	0.5 rps
Nebulization room temperature	2 °C
He gas flow rate	4.0 mL/min
Energy discrimination	-4.0 V
Signal collection mode	TRA

S3. Synthesis and characterization of CdTe QDs

The aqueous dispersions of L-GSH/L-Cys-capped CdTe QDs mainly used in this study were synthesized with certain modifications to the literature procedures[1-3] to achieve nanocrystals with good chemical stability and high photoluminescence quantum yield (PLQY). Briefly, 1.0 mmol (0.1276 g) of Te powder and 3.0 mmol (0.1703 g) of KBH_4 were dissolved in 1 mL of water under a nitrogen environment, and the reaction mixture was left magnetically stirring at room temperature for approximately 1 h until a light wine-red or colorless clear solution appeared. The resulting KHTe solution was kept under nitrogen (N_2) before use. 2.0 mmol of $CdCl_2 \cdot 2.5H_2O$ (0.464g), 0.6 mmol (0.193g) of L-GSH and 2.0 mmol (0.243 g) of L-Cys were dissolved in 400 mL of Millipore water, in the given order. This solution was loaded into a 500-mL glass three-neck flask. The pH of the solution was adjusted to approximately 9 until the solution become clear by the dropwise addition of 1 M NaOH solution with stirring. In the early stages, the formation of white precipitate occurred due to the rapid decrease of L-GSH and L-Cys solubility. The reaction mixture was thoroughly degassed at room temperature by bubbling N_2 for 30 min. The freshly prepared KHTe solution was then rapidly added to the stirred reaction system at room temperature. Then, the mixture was heated to 96 °C and refluxed for approximately 60 min under a slow N_2 flow to achieve the aqueous dispersions of L-GSH/L-Cys-capped CdTe QDs. During the synthesis, aliquots of the reaction solution were removed at regular intervals (5−10 min) and absorption spectra and emission spectra were taken to monitor the growth of the nanocrystals. After the growth time, the mixture was cooled quickly and stored at 4 °C. Before the animal experiment, the dispersion was purified by adding ethanol to the QDs solution with a 3:1 volume ratio. After ultracentrifugation (5 000 rpm) for 5 min, the precipitation was redissolved in 10 mM Na_2HPO_4 (pH 9.0) solution and then sonicated for 5 min at 50 W/60 Hz (KQ3200E, Kunshan instrument Co., Ltd, Kunshan, China). This process was repeated three times to ensure the removal of the residual coexistent ions and the absence of L-GSH and L-Cys.

The UV−visible absorption spectra of CdTe QDs were determined using a UV–Vis spectrophotometer (UV-2600, Shimadzu, Japan). The emission spectra were acquired using a spectrofluorometer (RF-5301pc, Shimadzu, Japan) at 450 nm excitation and room temperature using a 150 W xenon lamp as the excitation source. The excitation and emission slits were both 3 nm wide. All optical measurements were carried out in a 1 cm path length quartz cuvette with a screw cap for anaerobic work at room temperature under ambient conditions. AFM image was carried out with an AFM (Nanoscope 3D, Nikon, Japan) by depositing one of a water suspension

of the sample onto a mica surface (Quality V1, Plano GmbH, Wetzlar, Germany) and drying in the glass desiccator at room temperature under vacuum. Additionally, the monodispersity and stability of CdTe QDs in aqueous solution was estimated using a Malvern Zetasizer (Nano ZS90, Worcestershire, United Kingdom) by slowly injecting the zeta cell with the water diluted solution of CdTe QDs using a syringe until the entire U cell was filled with the sample without air bubbles and loaded it into the machine.

S4. Optimization of CZE separation conditions of CdTe QDs, Cd^{2+} and TeO_3^{2-} in different matrices

Electrophoresis separation was performed on a 7100 CE system coupled to the 8800 ICP-MS Triple Quad. The mixture samples consisting of CdTe QDs, Cd^{2+} and TeO_3^{2-} in buffer solution, cell culture solution (Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% penicillin/streptomycin) and serum sample (normal rat serum) were hydrodynamically injected into the capillary, respectively. On the basis of temperature, applied voltage and capillary length, as described above, the stable separation conditions were optimized by changing multiple factors, including the buffer composition and concentration, buffer solution pH, surfactant type, the ionic strength of solution, additives, etc.

S5. Quantification of CdTe QDs, Cd^{2+} and TeO_3^{2-} by CZE-ICP-MS

The calibration curve was prepared by plotting the peak area against the concentration of a series of Cd^{2+}/TeO_3^{2-} mixture standard solutions (the concentration of each ion in 12 mixed standard solutions was 1.0, 2.0, 5.0, 10, 20, 50, 100 200, 500, 1000, 2000 and 5000 μg/L, respectively) prepared in 40 mM BSB (pH 9.0). In addition, considering effect of the sample matrix on the determination and the separation of CdTe QDs, Cd^{2+} and TeO_3^{2-}, 2 mg/mL HSA, 2 multiples of dilution of cell culture solution, and 20 multiples of dilution of normal rat serum was added to the mixture standard solutions for the buffer solution, cell culture solution, and serum sample, respectively. Each standard and sample solution was injected in triplicate into the CZE-ICP-MS system. The simultaneous quantification of CdTe QDs, Cd^{2+} and TeO_3^{2-} in the above different matrices were performed by the external calibration curves Cd^{2+} and TeO_3^{2-}. At the same time, the analytical performance of the developed CZE-ICP-MS method, consisting of the external calibration curve, the limit of detection (LOD), the precision and the accuracy of the developed method, was evaluated under the optimal separation conditions. On this basis, two

commercial CdTe QDs, called CdTe-NH_2-560±5 QDs and CdTe-COOH-580±5 QDs (from a Chinese supplier), were analyzed by the developed CZE-ICP-MS.

S6. Acid digestion and CZE-ICP-MS determination of CdTe QDs

In addition, in order to compare and further verify the quantization method as described above, the concentration of CdTe QDs was also determined by the CE-ICP-MS after the sample was digested using a microwave digestion system (CEM Mars 5Xpress, Matthews, NC, USA) for complex matrix samples and concentrated HNO_3 for simple matrix samples. Acid digestion and CZE-ICP-MS determination of the CdTe QDs are described as follows: First, DMEM containing 10% FBS and 1% penicillin/streptomycin was mixed with a certain volume of diluted purified CdTe QDs solution as the cell culture solution sample. A certain volume of diluted purified CdTe QDs solution was added to normal rat serum as the serum sample. Then, concentrated HNO_3 and concentrated H_2O_2 were added to two complex matrix samples described above. After mixing thoroughly and leaving the samples for 1 h at room temperature, the mixture was microwave digested using a program as follows: ramp to 120 °C (800 W) in 10 min and hold for 10 min, followed ramp to 180 °C (1600 W) within 5 min and hold for 20 min. The digested solution was evaporated to remove superfluous acid and diluted with BSB (pH 9.0), in which the final concentration of BSB is 40 mmol/L. The quantification of Cd^{2+} and TeO_3^{2-} species of digested CdTe QDs was performed in accordance with the procedure described above. For the simple matrix samples, such as the synthesized CdTe QDs or commercial CdTe QDs solution, the sample was mixed with concentrated HNO_3 (the ultimate volume concentration is 10%) and mechanically shaken (250 rpm) for 2 h at room temperature instead of microwave digestion. After that, the acid-driving and CE-ICP-MS quantification method is the same as above.

S7. Serum pharmacokinetics of CdTe QDs in rats

All the animal experiments were performed in compliance with the institutional ethics committee regulations and guidelines on animal welfare with the approval of the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University (ethical review number: 2011-X-072). Male Wistar rats that weighed 200−220 g were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were housed under normal laboratory conditions with free access to standard rodent food and water. They were kept on a 12 h light/dark cycle. After acclimation, a total of 10 male rats were randomly divided into two

groups, that is, an experimental group and a control group. Each group contained five rats. The CdTe QDs solution was prepared by dissolving purified CdTe QDs that were synthesized in this study in normal saline. The experimental group was injected with CdTe QDs after purification through a tail vein at the dose of 5.0 μmol/kg body weight. The control group was injected with an equal volume of normal saline (6.25 mL/kg) in the same way. After dosage, at each time point (5, 15, 30 min, 1, 3, 6, 12 h, 1, 2, 3, 4, 5, 6, 7 and 8 d) 0.4 mL blood samples were collected from the tail vein of the rat in a 0.5-mL centrifuge tube. Then, the blood samples were centrifuged at 4000 rpm for 10 min to collect the serum, which was stored at a temperature not exceeding -20 °C. The concentrations of CdTe QDs, Cd^{2+} and TeO_3^{2-} in serum samples were determined by the CZE-ICP-MS method developed in this study.

S8. Statistical data analysis

Statistical analysis was performed using the Student's *t* test by SPSS 17.0 (SPSS Inc.). The *p* values <0.05 were considered statistically significant for all statistical analyses. The data are expressed as the mean ± the standard deviation (SD). The pharmacokinetic parameters of CdTe QDs in the serum were calculated using DAS 2.1 software.

Results and discussion

S9. Influence of pH value of BSB as the running buffer and the sample buffer on the separation of CdTe QDs，Cd^{2+} and TeO_3^{2-}

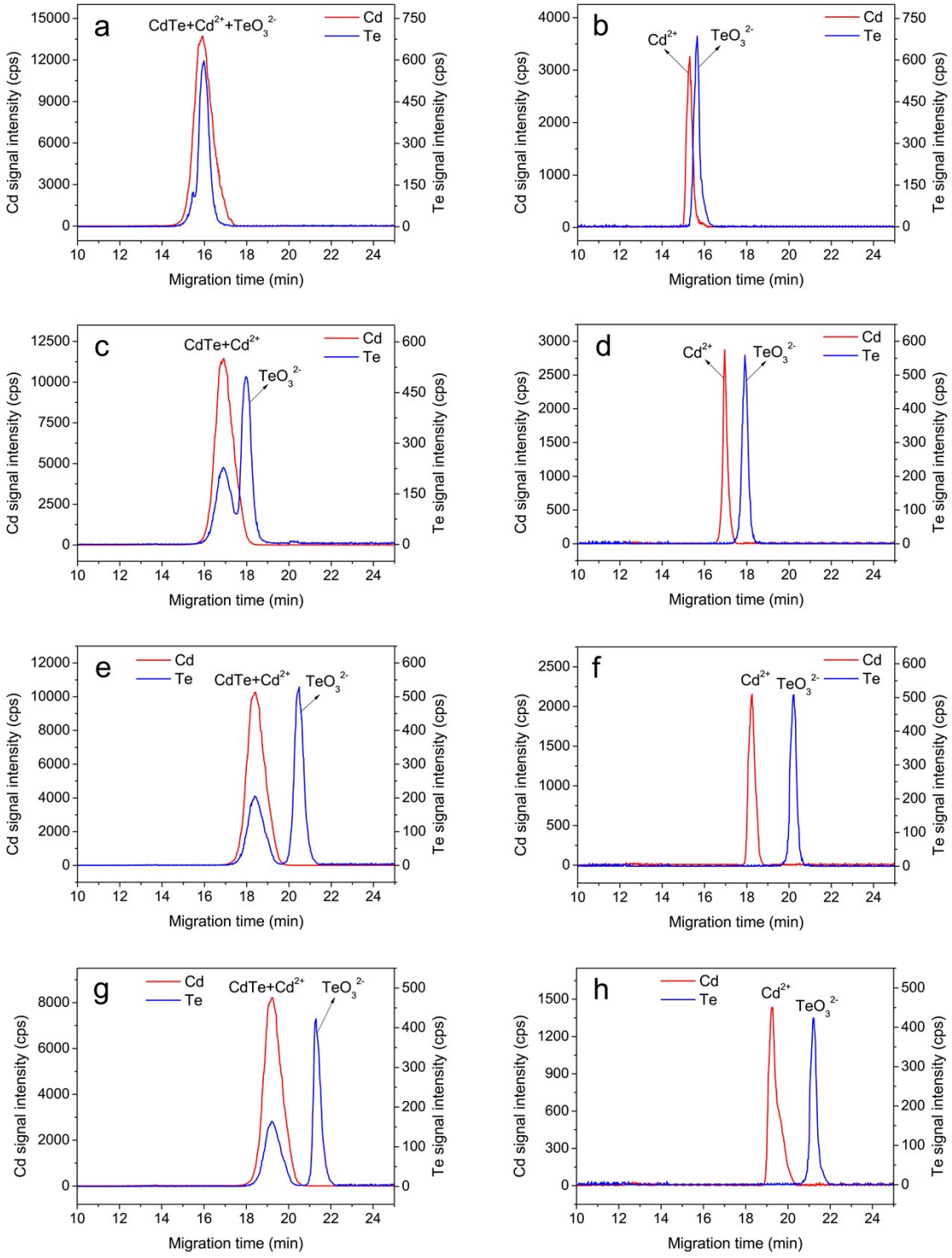

Figure S1 Electropherograms of (a, c, e, g) the mixture sample consisting of 40 μM CdTe QDs, 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS. Electropherograms of (b, d, f, h) the mixture sample consisting of 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS. The running buffer and the sample buffer are both 40 mM BSB with a pH value of (a, b) 8.4, (c, d) 9.0, (e, f) 9.3 and (g, h) 9.6, respectively.

S10. Influence of pH value of PB as the running buffer and the sample buffer on the separation of CdTe QDs，Cd^{2+} and TeO_3^{2-}

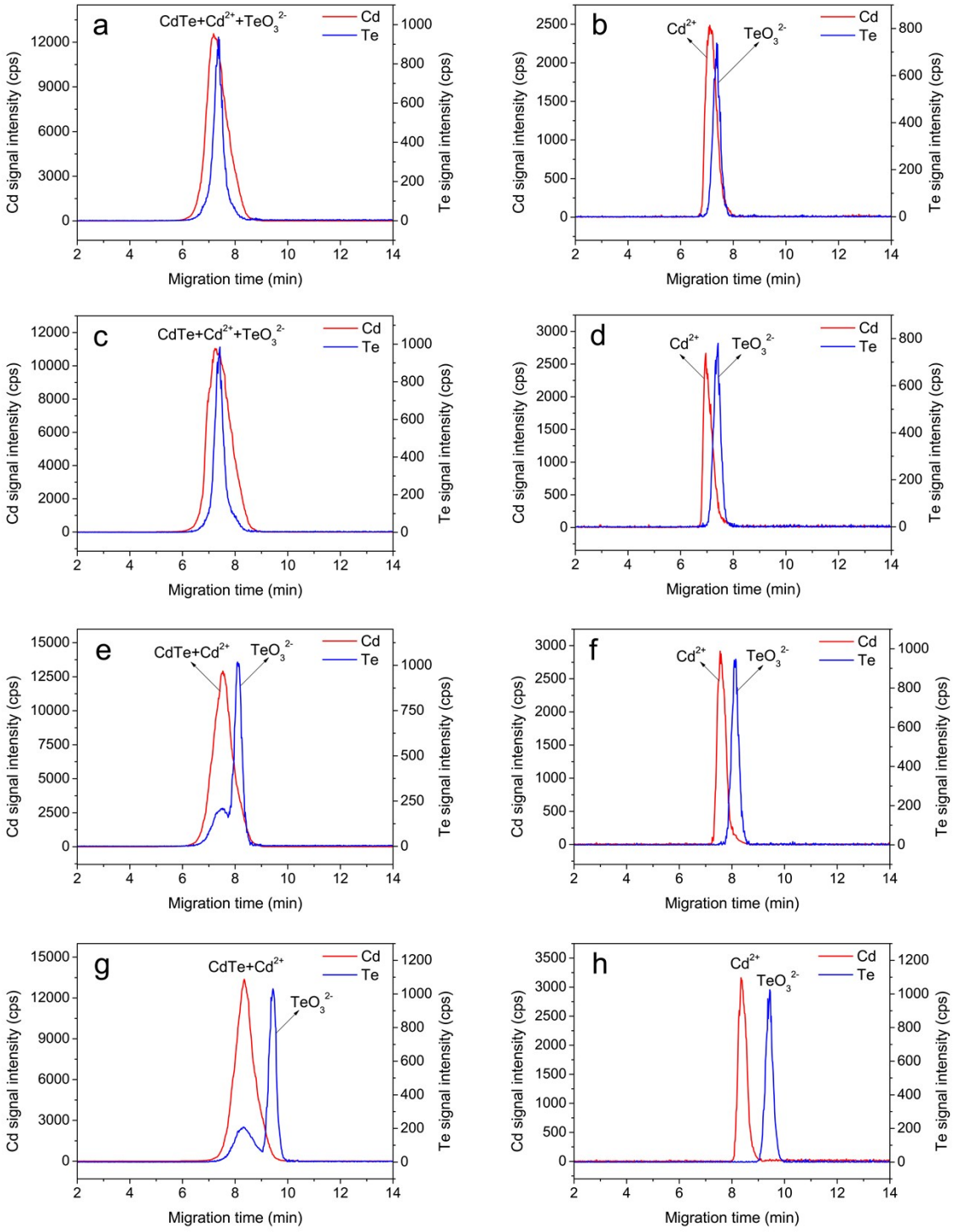

Figure S2 Electropherograms of (a, c, e, g) the mixture sample consisting of 40 μM CdTe QDs, 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS.

Electropherograms of (b, d, f, h) the mixture sample consisting of 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS. The running buffer and the sample buffer are both 30 mM PB with a pH value of (a, b) 8.0, (c, d) 8.2, (e, f) 8.4 and (g, h) 9.0, respectively.

S11. Influence of BSB (pH 9.0) concentration on the separation of Cd^{2+} and TeO_3^{2-}

The influence the concentration of BSB (pH 9.0) as the sample buffer on the retention performance of Cd^{2+} and TeO_3^{2-} was investigated by CZE-ICP-MS with 30 mM Na_2HPO_4 (pH 9.0) as the running buffer. The results in Figure S3 shows a delay of migration time of the Cd^{2+}-BSB complex and TeO_3^{2-}, which could be ascribed to an increase of the solution ionic strength with BSB concentration, and in turn, a decrease in EOF velocity. Furthermore, as shown in Figure S3h, narrow and symmetrical electrophoretic peak of both the Cd^{2+}-BSB complex and TeO_3^{2-} was achieved in the current analysis conditions.

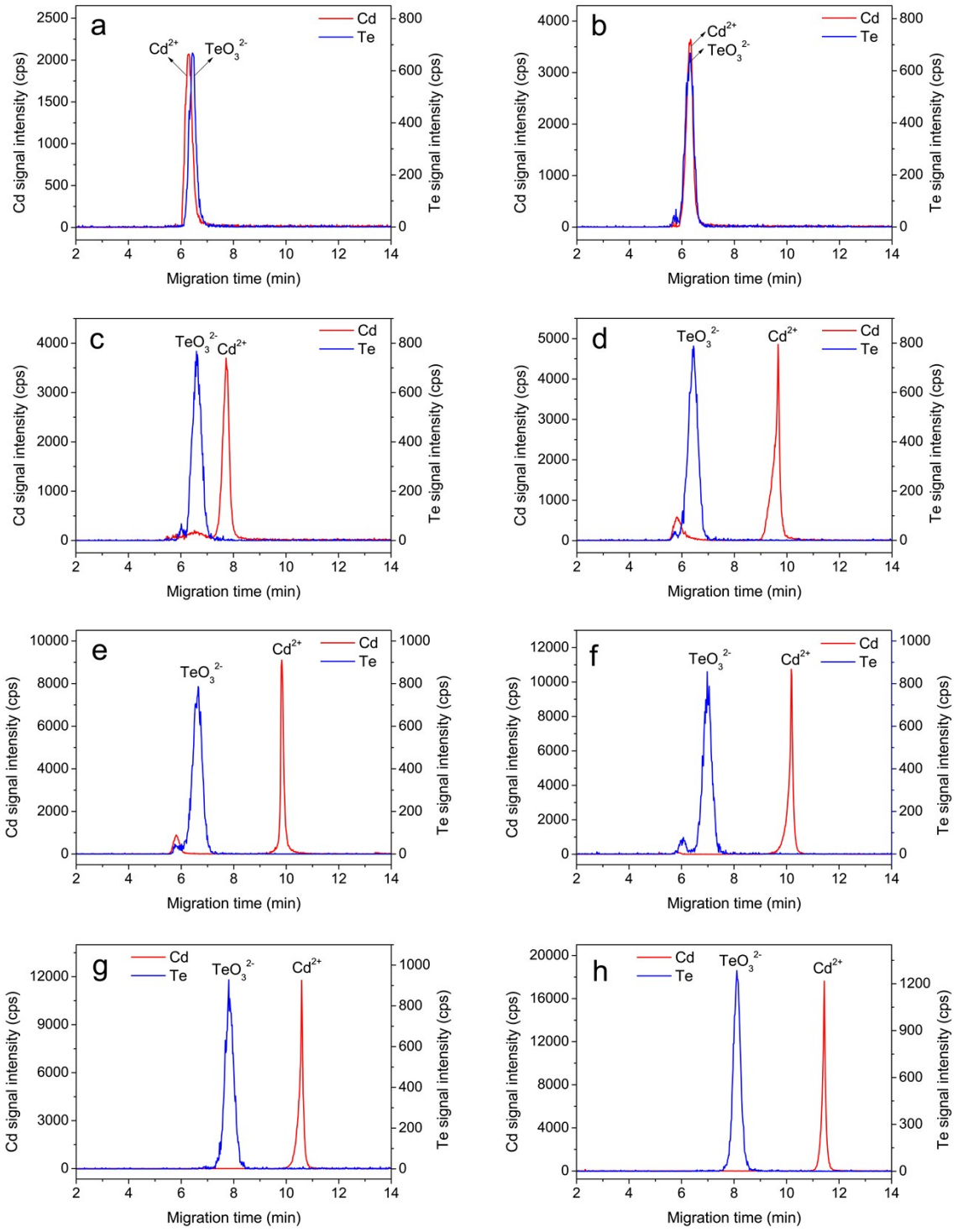

Figure S3 Electropherograms of the mixture sample consisting of 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by CZE-ICP-MS under different conditions. The running buffer is 30 mM Na_2HPO_4 (pH 9.0). 0.3% (v/v) Tween 20 and various concentrations of BSB (pH 9.0), (a) no BSB, (b) 1 mM BSB, (c) 2 mM BSB, (d) 4 mM BSB, (e) 8 mM BSB, (f) 16 mM BSB, (g) 32 mM BSB, (h) 40 mM BSB, were added to the sample solution.

S12. The influence of HSA concentration on the separation and method sensitivity of CdTe QDs, Cd^{2+} and TeO_3^{2-}

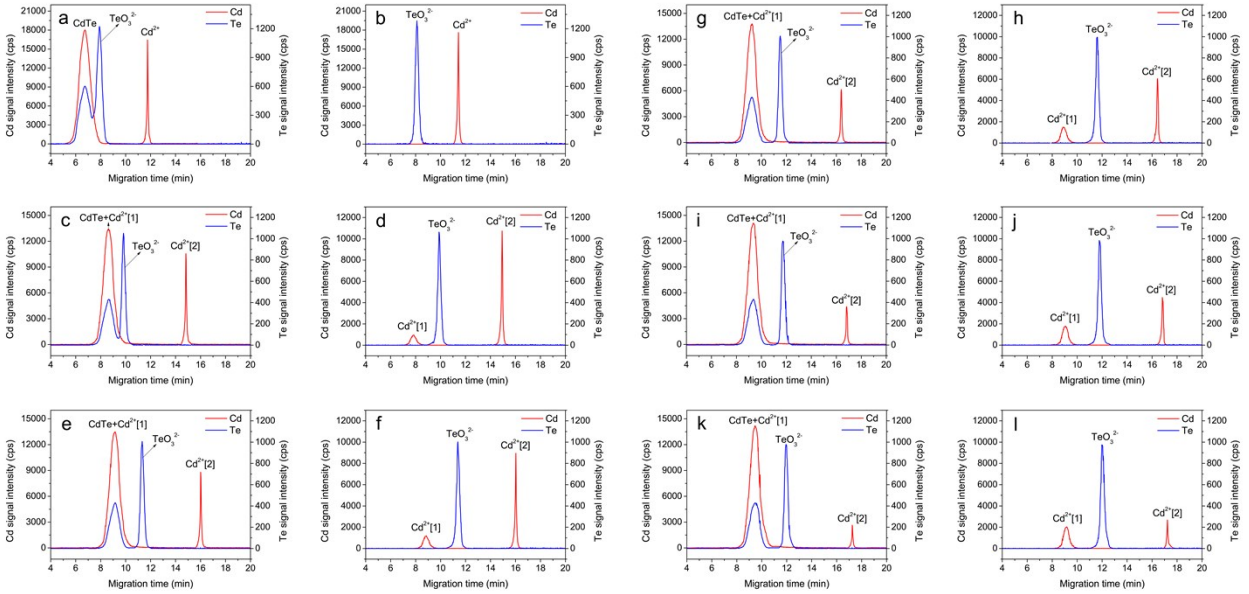

Figure S4 Electropherograms of (a, c, e, g, i, k) the mixture sample consisting of 40 μM CdTe QDs, 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS. Electropherograms of (b, d, f, h, j, l) the mixture sample consisting of 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by the CZE-ICP-MS. The running buffer is 30 mM Na_2HPO_4 (pH 9.0). 40 mM BSB (pH 9.0) and various concentrations of HSA, (a, b) no HSA, (c, d) 1 mg/mL HSA, (e, f) 2 mg/mL HSA, (g, h) 3 mg/mL HSA, (i, j) 4 mg/mL HSA, (k, l), 5 mg/mL HSA, were added to the sample solution.

S13. Influence of Na_2HPO_4 (pH 9.0) concentration on the separation of CdTe QDs, Cd^{2+} and TeO_3^{2-}

Under the current optimum conditions, the influence of the concentration of Na_2HPO_4 (pH 9.0) used as the running buffer on the separation of CdTe QDs, Cd^{2+} and TeO_3^{2-} was researched by CZE-ICP-MS. From the results shown in Figure S5, no Cd^{2+}[2] signal was observed for the lower concentration of Na_2HPO_4 (pH 9.0), while both the Cd^{2+}[2] signal intensity and resolution between CdTe QDs, Cd^{2+} and TeO_3^{2-} increased with Na_2HPO_4 (pH 9.0) concentration. However, for the increased concentration of Na_2HPO_4 (pH 9.0) of 40 mM, the phenomenon of a disordered electrophoretogram and an erratic electrophoretic current appeared. Thus, all subsequent experiments were performed with 30 mM Na_2HPO_4 (pH 9.0) as the running buffer. In addition, to ensure good reproducibility, the running buffer should be updated every five to eight samples.

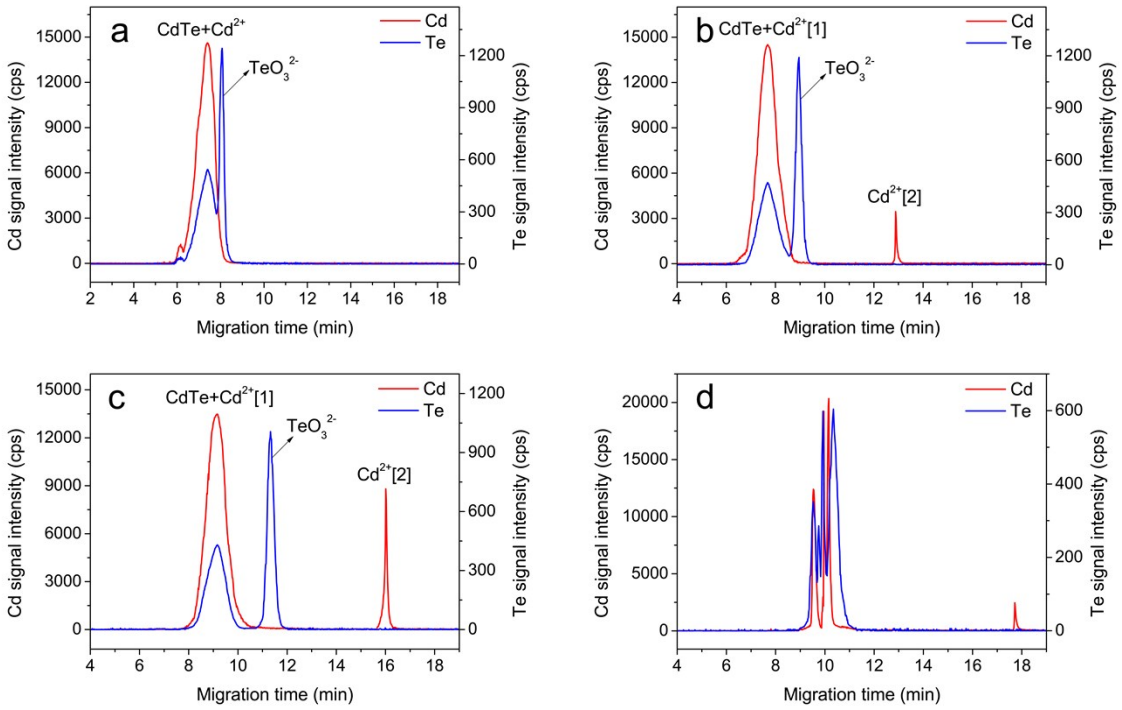

Figure S5 Electropherograms of the mixture sample consisting of 40 μM CdTe QDs, 0.5 mg/L Cd^{2+} and 0.5 mg/L TeO_3^{2-} in buffer solution obtained by CZE-ICP-MS. 2 mg/mL HSA and 40 mM BSB (pH 9.0) were added to the sample solution. The running buffer is 10 mM (a), 20 mM (b), 30 mM (c) and 40 mM (d) Na_2HPO_4 (pH 9.0), respectively

S14. Influence of the dilution factor of the serum sample on the analysis sensitivity of Cd^{2+}[2] by the CZE-ICP-MS

The quantification analysis results in Figure S6 showed that the proportion of the Cd^{2+}[2] in the Cd^{2+} raised with the increase of the dilution factor of serum sample. However, the absolute signal intensity of the Cd^{2+}[2] increased with the dilution factor in the range of 2−20, reached a maximum value at a dilution factor of 20, and then decreased with further increase of the dilution factor. Therefore, the serum sample was diluted 20 times as the optimum condition, and the other optimum conditions are consistent with previous experimental results.

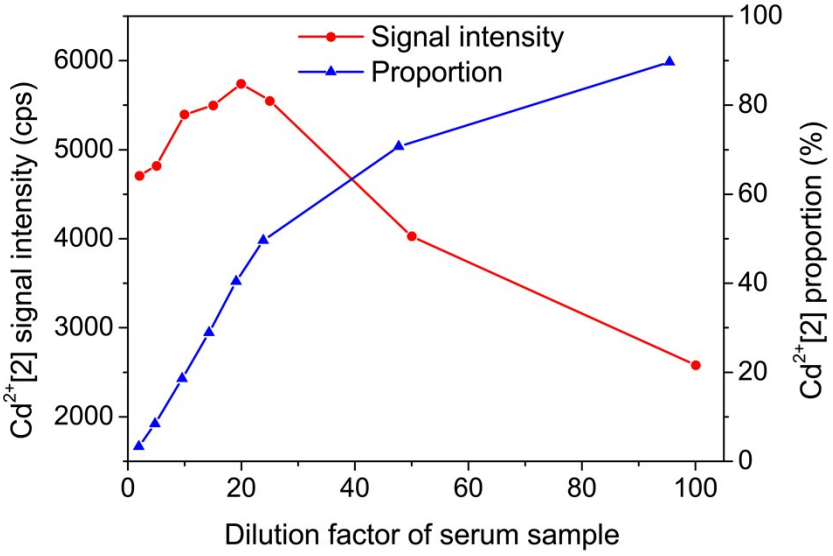

Figure S6 Influence of the dilution factor of the serum sample on the analysis sensitivity of Cd^{2+}[2] by the CZE-ICP-MS. The normal rat serum containing 1000 μg/L Cd^{2+} standard solution was diluted 2, 5, 10, 15, 20, 25, 50 and 100 times with BSB (pH 9.0), respectively, in which the final concentration of BSB is 40 mmol/L.

S15. Analytical performance of the developed CZE-ICP-MS method

The three calibration curves based on different Cd^{2+} species were prepared by plotting the peak area of Cd^{2+}[1], Cd^{2+}[2] and Cd^{2+} (Cd^{2+}[1] and Cd^{2+}[2]) against the concentration of Cd^{2+} in mixed standard solutions, respectively. A Cd^{2+} concentration of more than 1000 μg/L would result in a positive deviation for the calibration curve of Cd^{2+}[2] and a negative deviation for that of Cd^{2+}[1] in the cell culture solution and serum sample. The wide linearity range of the other two species containing the Cd^{2+} and TeO_3^{2-} in the cell culture solution and serum matrices and all ionic species (Cd^{2+}, Cd^{2+}[1], Cd^{2+}[2], and TeO_3^{2-}) in buffer solution was established in the entire concentration range (1−5000 μg/L) of the standard solution. A good linear relationship in the linear range was obtained with the linear correlation coefficient (r^2) in the range of 0.9966−0.9998 in all studied cases.

The limits of detection (LODs, 3σ) for TeO_3^{2-} and Cd^{2+} based on Cd^{2+}[2], which were estimated as three times the baseline noise, were 2.23 and 0.29 μg/L in the buffer solution, 5.34 and 0.86 μg/L in the cell culture solution, 3.23 and 0.53 μg/L in the serum sample, respectively. Since the total injected volume in CZE was in the nanoliter range, this corresponds to sub-femtograms for the Cd^{2+} and femtograms for the TeO_3^{2-} in the total detected mass. Consequently, the LODs of this developed method are quite satisfactory compared to other methods (> 100 fg).[4] Given that the LOD of Cd of CdTe QDs is lower than that of Cd^{2+}[1] or Cd^{2+}[2], the LOD of Cd of CdTe QDs would be regarded as the lower value of the LODs of Cd^{2+}[1] and Cd^{2+}[2]. The precision experiments showed that the relative standard deviation (%RSD) of all ionic species were in the range of 1.33−2.16 for the peak area and 0.14−0.33 for t_M in the buffer solution, 2.94−3.78 for the peak area and 0.43−0.58 for t_M in the cell culture solution, 2.19−4.43 for the peak area and 0.37−0.79 for t_M in serum sample, respectively, suggesting the good reliability of our method in quantifying Cd^{2+} and TeO_3^{2-} within a complex matrices sample. Meanwhile, the method accuracy was investigated by spiked recovery experiments. The CZE-ICP-MS electropherograms of the spiked recovery experiments indicate that the spiked recovery rates of both Cd^{2+} and TeO_3^{2-} were 99.15±7.55% for the buffer solution, 93.1±8.7% for the cell culture solution and 96.5±7.0% for the serum sample, respectively, which means that there is no interference from the diluent or the sample matrix. All the above performance parameters of the CZE-ICP-MS analysis are summarized in Table S2.

Table S2 Analytical performance of the developed CZE-ICP-MS method

sample matrices	species	linear regression equation, r^2	the upper limit of the linear range (mg/L)	LOD (μg/L)	spiked recovery (%)	RSD (%), n=6	
						peak area	migration time
buffer solution	Cd^{2+}[1]	Y=51.31X+368.4, 0.9998	>5.0	0.23	96.3～104.5	1.82	0.16
	Cd^{2+}[2]	Y=40.05X-37.21, 0.9985	>5.0	0.29	95.8～106.7	1.33	0.33
	Cd^{2+} [a]	Y=91.37X+331.2, 0.9993	>5.0	—	96.1～105.6	1.59	—
	TeO_3^{2-}	Y=16.27X+309.3, 0.9986	>5.0	2.23	91.6～105.3	2.06	0.14
cell culture solution	Cd^{2+}[1]	Y=23.69X+571.9, 0.9993	1.0	0.57	86.5～98.5	3.62	0.44
	Cd^{2+}[2]	Y=17.01X+241.4, 0.9966	1.0	0.86	84.4～97.2	2.94	0.58
	Cd^{2+}	Y=40.71X+813.3, 0.9992	>5.0	—	85.4～97.9	3.58	—
	TeO_3^{2-}	Y=6.317X-10.75, 0.9986	>5.0	5.34	90.1～101.8	3.78	0.43
serum sample	Cd^{2+}[1]	Y=51.24X+302.4, 0.9994	1.0	0.25	92.3～99.5	4.43	0.37
	Cd^{2+}[2]	Y=24.19X-20.81, 0.9945	1.0	0.53	90.4～103.5	3.28	0.79
	Cd^{2+}	Y=75.43X+281.6, 0.9997	>5.0	—	91.4～101.5	3.86	—
	TeO_3^{2-}	Y=11.62X-12.81, 0.9991	>5.0	3.23	89.5～100.9	2.19	0.41

[a] Cd^{2+} includes Cd^{2+}[1] and Cd^{2+}[2] species.

S16. The quantification of CdTe QDs with different sample pretreatment methods by the developed CZE-ICP-MS

Previous study results suggest that the NPs cannot be quantified directly by the online coupling ICP-MS system due to incomplete vaporization and size-dependent diffusion effects of the NPs.[5, 6] In the current study, the CdTe QDs with a diameter as small as approximately 5 nm was much smaller than those reported values (>20 nm in diameter reported), and the total injected volume in CZE was in the nanoliter range, which was considered very small compared to the sheath fluid. Therefore, we initially considered the feasibility that the quantification of the CdTe QDs-Cd and CdTe QDs-Te could be conducted by the external calibration curves of the Cd^{2+} and TeO_3^{2-}, respectively. To further verify the reasonableness of the above speculation, we quantified the CdTe QDs by CZE-ICP-MS with different sample pretreatment methods. Only a single CdTe QDs peak appears in the CZE-ICP-MS electropherogram without sample pretreatment (direct injection analysis) in different matrices. After the sample pretreatment (room temperature HNO_3 digestion or microwave digestion), the Cd^{2+}[1] peak, Cd^{2+}[2] peak and TeO_3^{2-} peak appear and CdTe QDs peak disappears, which suggests that the purification methods for the original CdTe QDs solutions have a good effect, and the Cd^{2+} and TeO_3^{2-} in solution after the sample pretreatment were considered to be from the degradation of CdTe QDs. The measured contents of CdTe QDs in different matrices with different methods are listed in in Table S3. The statistical analysis results showed that there was no statistically significant difference between the two measurement methods in the above three matrices (P >0.05). The results indicate that the CdTe QDs concentration (calculated as Cd and Te, respectively) in different matrices determined by the two methods agreed very well. Therefore, the above developed CZE-ICP-MS method is completely feasible for the simultaneous quantification of CdTe QDs, Cd^{2+} and TeO_3^{2-} in different matrices.

Table S3 The quantification results of CdTe QDs in different matrices with different sample pretreatment methods by the developed CZE-ICP-MS method (n=3)

sample matrices	Species [a]	detected by direct injection analysis ($\bar{x} \pm s$,mg/L)	detected after concentrated HNO_3 digestion ($\bar{x} \pm s$,mg/L)	detected after microwave digestion ($\bar{x} \pm s$,mg/L)	*t* value	*P* value
buffer solution	CdTe QDs-Cd	448.5±20.8	460.4±34.1	—	1.23	>0.05
	CdTe QDs-Te	128.2±10.5	139.7±15.4	—	1.56	>0.05
cell culture solution	CdTe QDs-Cd	451.3±30.4	—	472.4±25.9	0.73	>0.05
	CdTe QDs-Te	135.7±15.6	—	146.6±20.3	1.48	>0.05
serum sample	CdTe QDs-Cd	430.7±23.6	—	441.2±24.3	1.12	>0.05
	CdTe QDs-Te	124.5±17.3	—	127.5±12.4	0.59	>0.05

[a] CdTe QDs-Cd and CdTe QDs-Te represent the content of Cd and Te of CdTe QDs, respectively.

S17. Quality assessment of commercial CdTe QDs

Electropherograms of two commercial CdTe QDs are shown in Figure S4. The results of the quantitative analysis are shown in Table S4. As can be seen, three species containing CdTe QDs, TeO_3^{2-} and Cd^{2+}[2] in two samples were separated successfully, which were further verified by spiking Cd^{2+}/TeO_3^{2-} mixed standard solutions in three sample solutions, respectively. The spiked recoveries of Cd^{2+} and TeO_3^{2-} were in the range of 94.7−104.3% and 91.2−99.8%, respectively. The excellent recoveries revealed that the sample matrix showed no interference for the determination of CdTe QDs, Cd^{2+} and TeO_3^{2-}, and the quantification results are quite accurate. The results showed that the Cd^{2+} content in the original CdTe-NH_2-560±5 solution occupied approximately 64.0% of the total Cd (CdTe QDs-Cd and Cd^{2+}), and the content proportion of the TeO_3^{2-} in the original CdTe-NH_2-560±5 QDs solution was approximately 47.7% of the total Te (CdTe QDs-Te and TeO_3^{2-}). The analysis results showed that the CdTe-NH_2-560±5 QDs have exhibited severe degradation and/or the purification effect was not effective after synthesis. In addition, the molar ratio of Cd to Te for the CdTe-NH_2-560±5 QDs species was 2.96:1, which was significantly lower than that of the CdTe-NH_2-560±5 QDs solution (4.32:1), and possibly means that the release of Cd from CdTe QDs was faster than that of Te. Similar phenomena were found for the original CdTe-COOH-580±5 QDs solution. The Cd^{2+} and TeO_3^{2-} contents occupied approximately 39.3% of the total Cd and 3.3% of the total Te, respectively. The molar ratio of Cd to Te decreased from 3.77:1 for the original CdTe-COOH-580±5 QDs solution to 2.36:1 for the CdTe-COOH-580±5 QDs species.

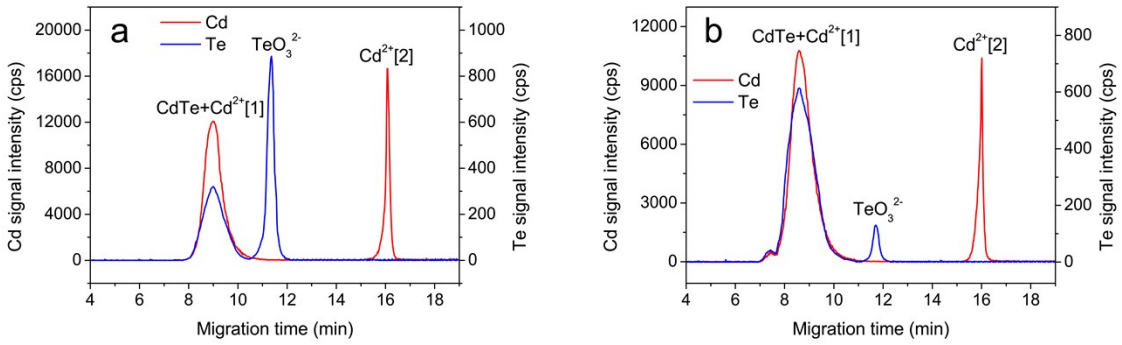

Figure S7 Electropherograms of (a) a commercial amino terminated CdTe QDs (CdTe-NH_2-560±5) and (b) a commercial carboxyl terminated CdTe QDs (CdTe-COOH-580±5) in buffer solution under optimal separation conditions by CZE-ICP-MS.

Table S4. The quantification results of commercial CdTe QDs using the developed CZE-ICP-MS method (n=3)

CdTe QDs	CdTe QDs-Cd ($\bar{x} \pm s$,mg/L)	Cd^{2+} ($\bar{x} \pm s$,mg/L)	CdTe QDs-Te ($\bar{x} \pm s$,mg/L)	TeO_3^{2-} ($\bar{x} \pm s$,mg/L)
CdTe-NH_2-560±5 QDs [a]	285.1±13.9	508.4±21.3	107.3±4.1	97.7±5.4
CdTe-COOH-580±5 QDs [b]	463.6±18.5	300.4±10.4	219.2±8.8	7.4±0.5

[a] The surface functional group and the maximum emission wavelength of CdTe QDs are NH_2 and 560±5 nm, respectively. [b] The surface functional group and the maximum emission wavelength of CdTe QDs are COOH and 580±5 nm, respectively.

S18. Serum pharmacokinetics of CdTe QDs

The concentration changes of CdTe QDs (the initial molar ratio of Cd/Te is 4.56±0.38), Cd^{2+} and TeO_3^{2-} in serum with time were investigated by the developed CZE-ICP-MS, as shown in Table S5. The results show that the concentrations of CdTe QDs-Cd and CdTe QDs-Te in serum continuously decrease with time throughout the metabolic process. After 7 days of exposure, the CdTe QDs-Cd and CdTe QDs-Te were not detected. In addition, the molar ratio of CdTe QDs-Cd to CdTe QDs-Te in serum was reduced from 4.50±0.24 to 0.79±0.37, which means that the surface of the CdTe QDs was oxidized, resulting in the formation of reduced Cd and the release of Cd^{2+}, which could be further demonstrated by the increase of Cd^{2+} concentration in the serum, described as follows. The Cd^{2+} concentration in the serum increased gradually within 3 hours after tail vein injection, and then decreased and remained at approximately 170 μg/L during 1 to 4 days, which possibly can be ascribed to the dynamic equilibrium between the clearance and release of Cd^{2+} in serum. After 6 days, the Cd^{2+} concentration remained steady (approximately 69 μg/L), accompanied by the complete clearance of CdTe QDs, which is basically consistent with that of control group. Moreover, the TeO_3^{2-} in serum was undetectable throughout the 8-day period of observation, which might be attributed to the rapid distribution or exclusion of TeO_3^{2-} derived from degradation of CdTe QDs. Alternately, the CdTe QDs itself may not be able to release Te and be further oxidized to form TeO_3^{2-} oxyanions under the current conditions; the specific mechanism needs further study. In addition, the serum pharmacokinetic behavior of CdTe QDs-Cd and CdTe QDs-Te is apparently different. Except for the similar apparent volume of distribution of the central compartment (V1), the smaller elimination half-life time ($t_{1/2}$), the bigger area under serum concentration-time profiles (AUC) and the clearance rate (CL) based on CdTe QDs-Cd indicated that CdTe QDs-Cd is easier to be cleared from blood circulation than CdTe QDs-Te.

Table S5 Concentration−time profiles of different Cd and Te species in serum after intravenous injection of CdTe QDs (n=5)

Time	CdTe QDs-Cd ($\bar{x} \pm s$, μg/L)	CdTe QDs-Te ($\bar{x} \pm s$, μg/L)	molar ratio of Cd/Te of CdTe QDs	Cd^{2+} ($\bar{x} \pm s$, μg/L)	TeO_3^{2-} ($\bar{x} \pm s$, μg/L)
5 min	17059.04±336.09	4230.84±145.42	4.50±0.24	321.57±76.15	ND [a]
15 min	16992.51±331.73	4214.19±142.44	4.50±0.23	491.07±102.84	ND
30 min	16301.95±327.06	4058.18±76.78	4.48±0.17	510.13±115.32	ND
1 h	13297.27±538.30	3710.27±101.74	4.00±0.26	599.34±128.91	ND
3 h	10850.74±386.47	3414.41±101.02	3.55±0.22	718.79±157.82	ND
6 h	9655.19±110.61	3123.62±180.17	3.45±0.24	389.64±104.14	ND
12 h	7748.87±475.81	2607.84±141.67	3.32±0.37	244.48±67.23	ND
1 d	3607.60±979.85	1727.48±287.07	2.33±0.98	182.33±54.53	ND
2 d	1736.01±447.56	835.11±131.99	2.32±0.94	153.93±60.09	ND
3 d	555.08±176.17	316.81±53.72	1.96±0.97	179.64±70.33	ND
4 d	248.07±70.01	186.03±33.48	1.49±0.63	167.76±66.49	ND
5 d	64.60±19.23	60.03±10.69	1.19±0.58	94.49±45.21	ND
6 d	25.6±3.48	35.82±10.21	0.79±0.37	75.56±44.75	ND
7 d	14.29±5.11	ND	—	69.52±49.23	ND
8 d	ND	ND	—	68.54±38.65	ND

ND [a] means undetectable.

Notes and references

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