Electronic Supplementary Information

Selective reduction-based highly sensitive and homogeneous detection of iodide and melamine using chemical vapour generation-atomic fluorescence spectrometry

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Materials and Instrumentation

Materials: All oligonucleotides were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China; Table S1). Stock solutions of the oligonucleotides were prepared according to the manufacturer's instruction and stored at -20 °C. The concentration was quantified by UV absorption at 260 nm. All reagents used were of the highest purity available but at least of analytical grade. The 1000 mg L^{-1} inorganic mercury (Hg (II)) stock solution was purchased from the National Research Center for Standard Materials (NRCSM) of China. High purity NaI, $SnCl_2$, $Mg(NO_3)_2$, HCl and NaOH were purchased from Kelong Chemical Factory (Chengdu, China). Melamine and other chemicals were purchased from Solarbio Technology Co., Ltd (Beijing, China). All working solutions were prepared with phosphate buffered saline (PBS, 10 mM, pH 7.4). High-purity argon gas used as carrier gas and shielded gas was obtained from Qiaoyuan Gas Company (Chengdu, China). High purity deionized water (18.2 MΩ cm) from a water purification system (PCWJ-10, Chengdu Pure Technology Co., Chengdu, China) was used throughout this work.

Table S1 Sequences of oligonucleotides used in the experiment.

Name	Sequence (5'-3')
P1	GCTTTTTTGCACGTACGTAAGCTTTTTTGC
P2 -1	GCAGCTTTTTTCGAC
P2-2	GTCGTTTTTTGCTGC
P3	GTTTTTTCCCCCCACCCCCCACGTTTTTTC

P4	TTTTTTTTTTTTTTTTTTTTTTTTTTTTT

Instrumentation: All fluorescence experiments were conducted on an AFS-2202 (Beijing Haiguang Instrument Co., Beijing, China) with a high intensity mercury hollow cathode lamp (253.7 nm). The instrumental optimized parameters are showed in Fig. S1 and summarized in Table S2. UV-visible absorption spectra were recorded with a Hitachi U-1750 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). The pH was measured by a model FE20 pH meter (Mettler Toledo, Shanghai, China). Temperature was controlled with a Blue Pard THZ-100 incubation shaker. Agilent 8900 ICP-MS was used to detect I^- in water sample (Agilent Technologies, Tokyo, Japan). Liquid chromatography coupled with TSQ Quantum Ultra Mass Spectrometer (LC-MS) was used to detect melamine in milk and milk powder samples (Thermo Fisher Scientific, Waltham, MA, USA).

Table S2 Operating parameters of atomic fluorescence spectrometer

Parameters	Value
Sampling time /s	8
PMT high voltage /V	-250
Carrier gas flow rate /mL min^{-1}	300
Shielded gas flow rate /mL min^{-1}	800
Observation height /mm	8
Reading time /s	10
Delayed time /s	1
Hg hollow cathode lamp current /mA	30
Pump rotation /r min^{-1}	100

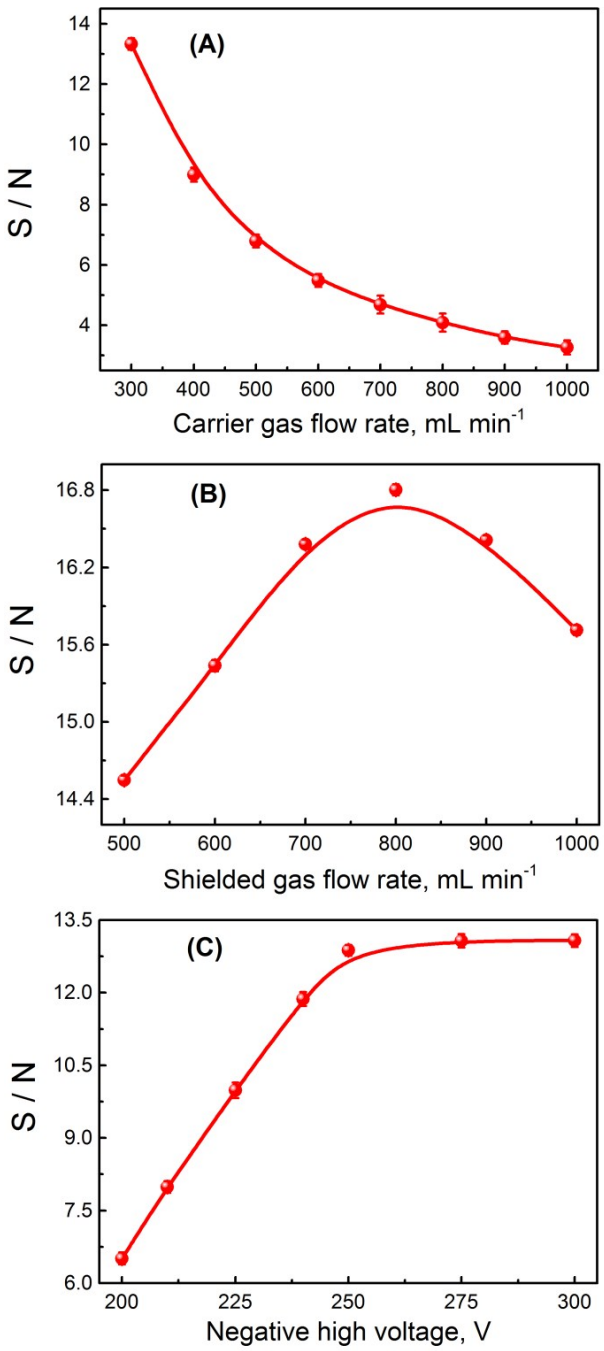

Fig. S1 Optimization of AFS conditions. (A) Carrier gas flow rate, (B) shielded gas flow rate, and (C) negative high voltage. With 40 nM Hg^{2+} solution. S/N: signal to noise.

General procedure

For I^- detection: As shown in Scheme 1, for the single-strand DNA (P1), 100 μL of 1 μM P1 and 200 μL of 2.5 μM Hg^{2+} were added to 600 μL phosphate buffered saline (PBS, 10 mM, 10 mM Mg^{2+}, pH 7.4) to form T-Hg^{2+}-T hairpin structure. After 2 hour incubation at room temperature, 100 μL different concentration of I^- solution and water sample were added to the above mixture system to perform the competitive reaction between the T-Hg^{2+}-T hairpin structure and I^- for 1 hour. Then the solution was diluted to 10 mL with ultrapure water. Finally, the fluorescence intensity was detected by AFS for I^- quantitative analysis. The procedure of double-strand DNA (P2-1 and P2-2) was the same as P1 just instead of P1 with the P2-1 and P2-2 with the same concentration and volume. All the measurements were repeated three times at least.

For melamine detection: as shown in Fig. 3A. Briefly, reaction mixtures of known concentration of Hg^{2+} and the probe ssDNA (P3 and P4, sequences shown in Table S1) were incubated at room temperature for 2h (for P3 as capture probe) or 1 h (for P4 as capture probe) to form the T-Hg^{2+}-T complex structure. Then the melamine of different concentration, different interferences and real samples were added into the aliquots of the above mixtures for 2.5 h (for P3 as probe) or 2 h (for P4 as probe) to perform the reaction between melamine and probe sequence-Hg^{2+} complex and release the free Hg^{2+}. Subsequently, the above reaction mixtures were diluted to 10 mL of with ultrapure water. Finally, the mercury signals were obtained from the diluted solutions and recorded by AFS for melamine quantitative analysis.

Sample preparation

The water samples collected from the lotus pond on the campus of Sichuan University, and filtered through a 0.22 μm filter and subsequently stored in 4 °C before analysis. Milk samples were prepared following a previous method with a minor modification. [1] Briefly, 5.0 mg of milk powder or 5.0 mL of raw milk was placed in a 10 mL centrifuge tube, and 1.5 mL of 2 M trichloroacetic acid was introduced. After ultrasonication for 10 min, the mixture was centrifuged at 6000 rpm for 10 min. The supernatants were adjusted to pH 7.0 with NaOH, filtered with 0.22 μm membrane and diluted 100-fold with PBS before use.

Optimized of sensing conditions for I^-

Formation of T-Hg^{2+}-T: the time and ratio of forming the T-Hg^{2+}-T was similar to the previous results, the time was 2 h and the ratio of capture DNA and Hg^{2+} was 1:5, as shown in Fig. S2A and S2B. Furthermore, Mg^{2+} in PBS played an important role in stabilizing the T-Hg^{2+}-T structure and accelerating the reaction rate. As shown in Fig. S2C, Mg^{2+} concentrations in PBS of 10 mM for P1 and 5 mM for P2 were selected for further study.

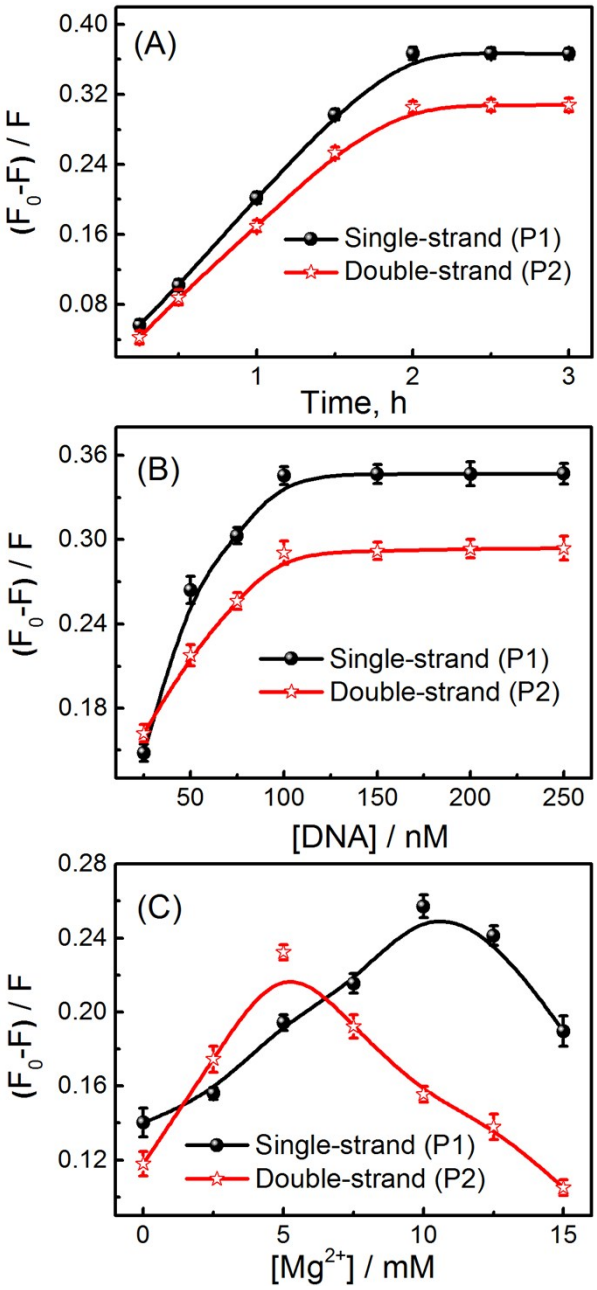

Fig. S2 Dependence of mercury fluorescence signal upon the formation T-Hg^{2+}-T structure parameters. (A) Incubation time; (B) concentration of capture DNA; and (C) concentration of Mg^{2+} in PBS. F_0 and F represent the fluorescence intensities of the solution containing 50 nM Hg^{2+} in the absence and presence of 10 nM probe DNA, respectively.

Selective reduction: the pH of the diluted solution and concentration of the $SnCl_2$ reductant were optimized. The pH 7.0 (use ultrapure water) was chosen for further

work, as shown in Fig. S3A. The concentration of 0.15% and 0.10% (m/v) $SnCl_2$ for P1 and P2 were ultimately selected for subsequent experiments respectively, based on a compromise between signal intensity and selective reduction HgI_2 (or HgI_4^{2-}) and T-Hg^{2+}-T, as shown in Fig. S3B.

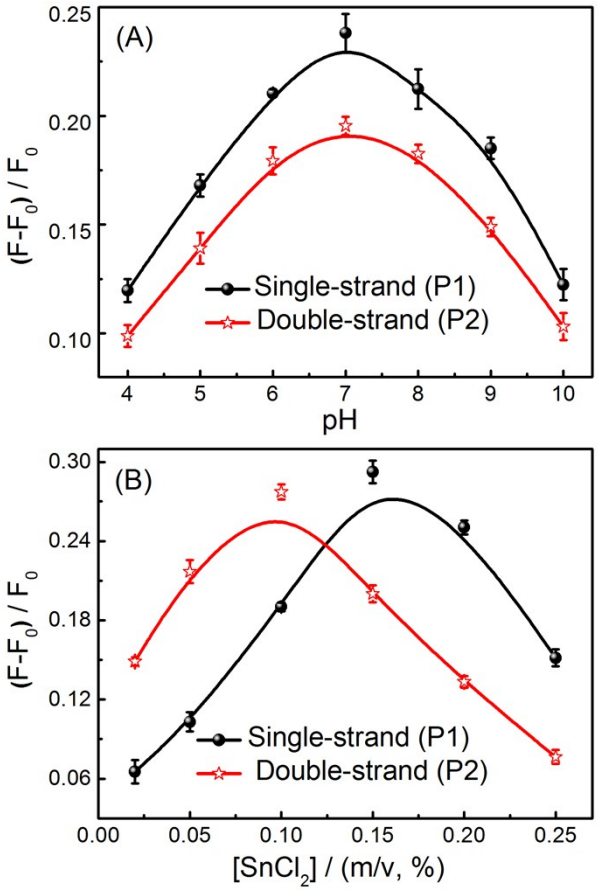

Fig. S3 Dependence of mercury fluorescence signal upon the selective reduction parameters. (A) pH of the diluted solution, and (B) concentration of the $SnCl_2$ reductant. F_0 and F represent the fluorescence intensities of the solution containing 50 nM Hg^{2+} and 10 nM probe DNA, in the absence and presence of 1 μM I^-, respectively.

Competition reaction time: the appropriate competition reaction time was also studied for the competition reaction between I^- with the T-Hg^{2+}-T complex. The ($F-F_0$)

/ F_0 increased rapidly with increasing incubation time from 0 to 1 h and then leveled off after 1 h (as shown in Fig. S4). In the view of time efficiency, an incubating time of 1 h was adopted for the competition reaction.

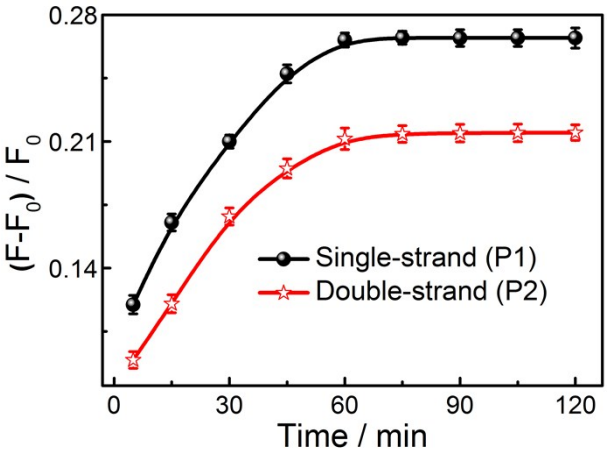

Fig. S4 Dependence of mercury fluorescence signals upon competition reaction time. F_0 and F represent the fluorescence intensities of the solution containing 50 nM Hg^{2+} and 10 nM probe DNA, in the absence and presence of 1 μM I^-, respectively.

The calculation of limit of detection (LOD): [2]

The LOD was estimated without target (I^- or melamine) giving AFS signal three times standard deviation higher than the background signal. The standard curve of I^- and melamine was plotted as: $\boldsymbol{Y = A\,\mathrm{Log}C + B}$ **(1)**

Where A and B are the variable obtained via least-square root linear regression for the signal-the logarithm value of target concentrations curve for variable Y representing the AFS signal at target concentration of *C* (M / mol L^{-1}).

When: $\boldsymbol{Y = Y_{blank} + 3SD}$ **(2)**

Where SD is the standard deviation (n=11) and Y_{blank} is the AFS signal of a blank

sample (without target).

The LOD was calculated as

$$LOD = 10^{[(Y_{blank} + 3SD) - B] / A} \quad (3)$$

Table S3 Analytical performance in comparison with those of other assays for I^-

Method	LOD; Linear range	Real sample	Reference
Colorimetric (Ag NPs)	65 nM; 0.1-25 μM	Urine and river water	[3]
Chemiluminescence	12 nM; 10 nM-10 μM	Lake water	[4]
Fluorescence (Au NCs)	0.3 nM; 0.001-6 μM	Urine	[5]
Fluorescence (Cu NCs)	15 nM; 0.05-40 μM	Kunming mice	[6]
Fluorescence (Ag NCs)	0.5 nM; 0.001-10.0 μM	Urine	[7]
Fluorescence (Dye)	22.6 nM; 0.01-2.0 μM	Running water	[8]
Electrochemical	25 μM; 10 μM-0.1 M	Sea, tap and river water	[9]
HPLC	0.2 ng/μL ; 0.2-100 ng/μL	Seaweed products	[10]
SERS	0.01 μM; 0.01 μM-2.0 μM	not mentioned	[11]
FAAS	2.75 μg L^{-1}; not mentioned	Milk	[12]
ICP-MS	0.002 μg/g; not mentioned	Soybean	[13]
CVG-AFS	0.25 nM; 1 nM-10 μM	Pond water	This work
CVG-AFS	0.18 nM; 1 nM-10 μM	Pond water	This work

Table S4 Determination of I^- in real water sample using CVG-AFS and ICP-MS

			CVG-AFS mean		ICP-MS mean	
Probe	Detected	I^- added	I^- found [a]	Recovery, %	I^- found	Recovery, %
P1	ND[b]	10.0 nM	9.5 ± 0.1 nM	95	-	-
		1.0 μM	0.99 ± 0.04 μM	99	0.99 ± 0.03 μM	99%
		10.0 μM	10.2 ± 0.2 μM	102	9.95 ± 0.12 μM	99%
	ND	10.0 nM,	9.6 ± 0.1 nM	96	-	-

P2	1.0 μM	0.96 ± 0.05 μM	95	0.97 ± 0.02 μM	97%
	10.0 μM	9.9 ± 0.3 μM	99	10.1 ± 0.2 μM	101%

[a] Mean and standard deviation of results (n = 3), [b] not detected.

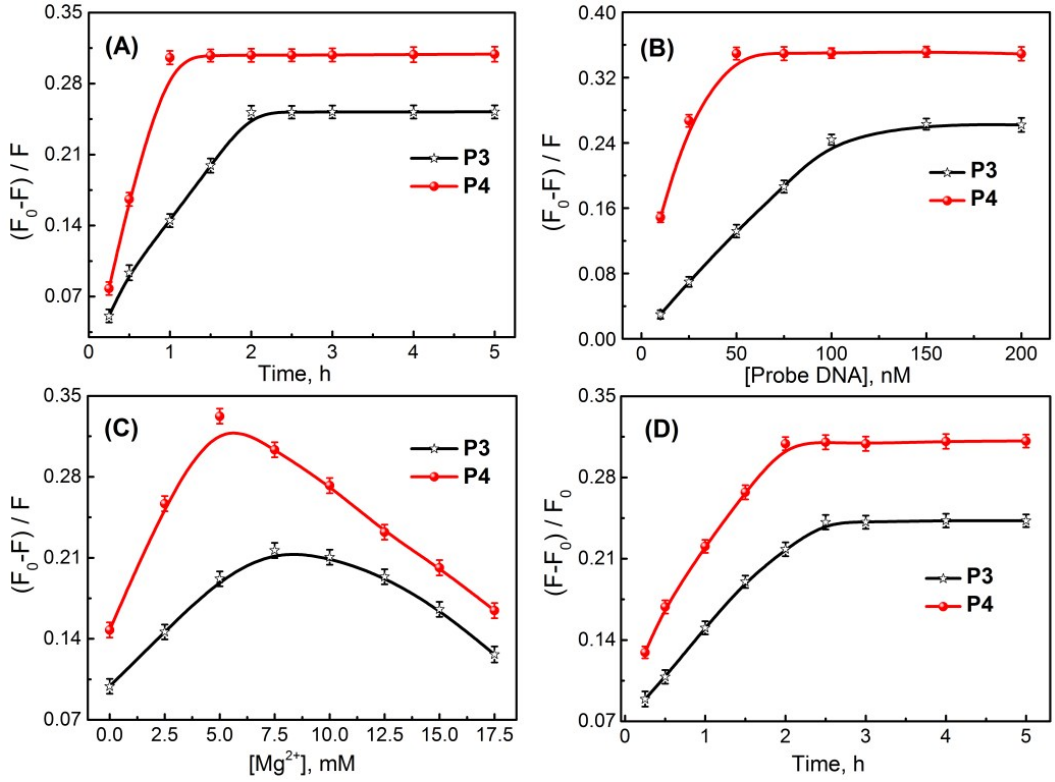

Fig. S5 Optimization of experimental conditions for melamine detection. (A) Incubation time, (B) ratio of the capture probe DNA (P3 and P4) and Hg^{2+}, (C) concentration of Mg^{2+} in PBS, and (D) The competitive reaction time.

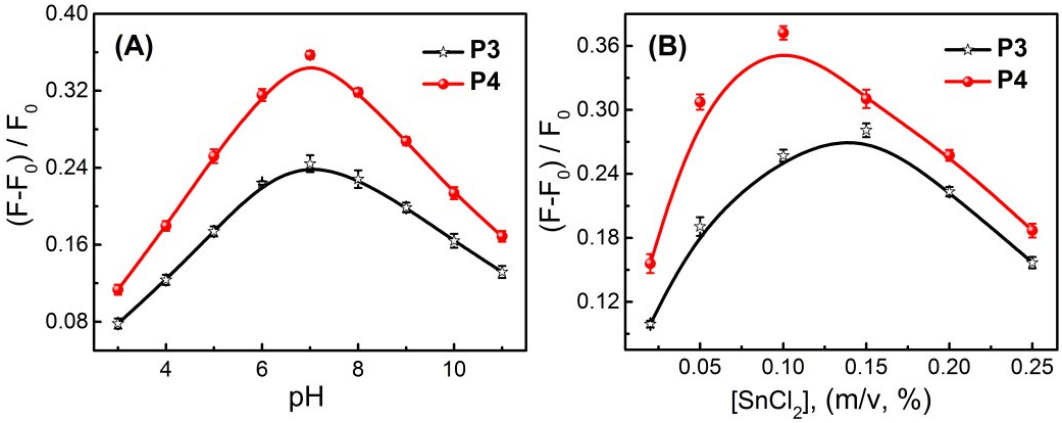

Fig. S6 Selective reduction conditions. (A) pH of the diluted solution, and (B) $SnCl_2$ concentration

Table S5 Comparison of different methods for the determination of melamine

Method	LOD; Linear range	Real sample	Reference
Visual (TMB)	0.08 μM; 0.5-100 μM	milk, milk powder	[14]
Colorimetric (Au NPs-TMB)	0.2 nM; 1 nM-0.8 μM	raw milk, milk powder	[15]
Fluorescence (FRET-Ag NPs)	23 nM; 0.1-40 μM	milk	[16]
Fluorescence (FRET-UCNPs)	18 nM; 32-500 nM	raw milk	[17]
Fluorescence (Ag NCs)	0.1 μM; 0.2-4 μM	raw milk, milk powder	[18]
Fluorescence (Cu NCs)	95 nM; 0.1 μM-6 μM	milk	[19]
Electrochemical	8.7 nM; 50 nM-500 μM	animal feed	[20]
SERS	8 nM; 10 nM-0.1 mM	milk	[21]
Personal glucose meter	0.33 μM; not mentioned	milk	[22]
CVG-AFS	0.2 nM; 1 nM-10 μM	raw milk, milk powder	This work
CVG-AFS	0.02 nM; 0.1 nM-1 μM	raw milk, milk powder	This work

Table S6 Analytical results of the melamine in milk and milk powder sample using the CVG-AFS and LC-MS

			CVG-AFS mean		LC-MS mean	
Probe	Sample	Added	Found[a]	Recovery, %	Found[a]	Recovery, %
P3	milk	10.0 nM	10.4 ± 0.2 nM	104%	-	-
		1.0 μM	0.95 ± 0.06 μM	95%	0.95 ± 0.04 μM	95%
		10 μM	9.9 ± 0.4 μM	99%	8.9 ± 0.3 μM	89%
	milk powder	10.0 nM	9.9 ± 0.2 nM	99%	-	-
		1.0 μM	1.05 ± 0.04 μM	105%	1.0 ± 0.05 μM	100%
		10.0 μM	10.3 ± 0.2 μM	103%	9.3 ± 0.2 μM	93%
	milk	1.0 nM	0.96 ± 0.04 nM	96%	-	-
		100 nM	93 ± 2 nM	93%	-	-

P4		1.0 μM	1.0 ± 0.1 μM	100%	0.92 ± 0.05 μM	92%
	milk powder	1.0 nM	0.90 ± 0.05 nM	90%	-	-
		100 nM	98 ± 2 nM	98%	-	-
		1.0 μM	0.89 ± 0.1 μM	89%	0.87 ± 0.06 μM	87%
[a] Mean and standard deviation of results (n = 3)						

References

1. X. F. Li, J. Li, H. Y. Kuang, L. Feng, S. J. Yi, X. D. Xia, H. W. Huang, Y. Chen, C. R. Tang and Y. L. Zeng, *Anal. Chim. Acta*., 2013, **802**, 82.
2. M. Ma, H. H. Yin, L. G. Xu, X. L. Wu, H. Kuang, L. B. Wang and C. L. Xu, *Chem. Commun*., 2014, **50**, 9737.
3. A. Niaz, A. Bibi, M. I. Zaman, M. Khan and A. Rahim, *J. Mol. Liq*., 2018, **249**, 1047.
4. T. Li, G. Liang and X. H. Li, *Analyst*., 2013, **138**, 1898.
5. W. L. Hou, Y. Chen, Q. J. Lu, M. L. Liu, Y. Y. Zhang and S. Z. Yao, *Talanta*., 2018, **180**, 144.
6. Z. Z. Chen, Y. X. Niu, G. Y. Cheng, L. L. Tong, G. L. Zhang, F. Cai, T. T. Chen, B. Liu and B. Tang, *Analyst*., 2017, 142, 2781.
7. L. P. Feng, Z. Z. Sun, H. Liu, M. Liu, Y. Jiang, C. Fan, Y. Y. Cai, S. Zhang, J. H. Xu and H. Wang, *Chem. Commun*., 2017, 53, 9466.
8. R. X. Zhang, P. F. Li, W. J. Zhang, N. Li and N. Zhao, *J. Mater. Chem. C*., 2016, 4, 10479.
9. M. Cuartero, G. A. Crespo, M. G. Afshar and E. Bakker, *Anal. Chem.,* 2014, **86**, 11387.
10. U. Nitschke and D. B. Stengel, *Food. Chem.,* 2015, 172, 326.
11. P. Pienpinijtham, X. X. Han, S. Ekgasit and Y. Ozaki, *Anal. Chem.,* 2011, **83**, 3655.
12. M. C. Yebra and M. H. Bollain, *Talanta*., 2010, **82**, 828.
13. J. T. P. Barbosa, C. M. M. Santos, L. dos Santos Bispo, F. H. Lyra, J. M. David, M. d. G. A. Korn and E. M. M. Flores, *Food. Anal. Methods.,* 2013, 6, 1065.
14. H. Hu, J. Y. Zhang, Y. Ding, X. F. Zhang, K. L. Xu, X. D. Hou and P. Wu, *Anal. Chem.,* 2017, 89, 5101.
15. P. J. Ni, H. C. Dai, Y. L. Wang, Y. J. Sun, Y. Shi, J. T. Hu and Z. Li, *Biosens. Bioelectron*.,

2014, **60**, 286.

16. L. Tang, S. Mo, S. G. Liu, Y. Ling, X. F. Zhang, N. B. Li and H. Q. Luo, *J. Agric. Food. Chem.,* 2018, 66, 2174.
17. Q. Q. Wu, Q. Long, H. T. Li, Y. Y. Zhang and S. Z. Yao, *Talanta.,* 2015, 136, 47.
18. P. S. Xie, Y. J. Zhan, M. Wu, L. H. Guo, Z. Y. Lin, B. Qiu, G. N. Chen and Z. W. Cai, *J. Lumin.,* 2017, 186, 103.
19. H. W. Zhu, W. X. Dai, X. D. Yu, J. J. Xu and H. Y. Chen, *Talanta*., 2015, **144**, 642.
20. X. J. Liu, M. M. Song and F. Li, *Sci. Rep.,* 2017, 7, 4490.
21. R. Wang, Y. Xu, R. J. Wang, C. Y. Wang, H. Z. Zhao, X. Q. Zheng, X. Liao and L. Cheng, *Microchim. Acta.,* 2017, 184, 279.
22. C. Gu, T. Lan, H. Shi and Y. Lu, *Anal. Chem.,* 2015, **87**, 7676.