Supplementary Information

DPD as an electron probe in ferrate oxidation: A novel spectrophotometric determination method and the fate of iron intermediates

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Text S1 Chemicals and Reagents.

N,N-Diethyl-p-phenylenediamine sulfate salt (DPD,≥ 98%) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS,≥ 98%) were obtained from Sigma-Aldrich. Phosphoric acid (H_3PO_4, AR), disodium hydrogen phosphate (Na_2HPO_4,AR), monosodium phosphate （NaH_2PO_4,AR), acetic acid (HAC,AR), sodium acetate (NaAC,AR), Magnesium chloride($MgCl_2$,AR), calcium chloride($CaCl_2$,AR), ammonium chloride ($NHCl_4$,AR), Sodium pyrophosphate($Na_4P_2O_7$,AR), sodium bicarbonate($NaHCO_3$,AR), sodium tetraborate ($Na_2B_4O_7$,AR), sodium hypochlorite(NaClO, contains available chlorine＞5%), hydrogen peroxide solution (H_2O_2,purity 30%), horseradish peroxidase, (HRP,RZ：>3.0 and activity：>300 units/mg) and phenol (AR) were obtained from Aladdin Reagents Co., Ltd. (Shanghai, China).

Phosphate buffer in the stocks solution of ferrate was used as a buffering system to maintain pH, as well as a chelating agent for Fe(III) to prevent the precipitation of its hydroxide compounds that could interfere with spectrophotometric analysis, and as a stabilizer to inhibit the self-decomposition of Fe(VI). The concentration of Fe(VI) in the stock solution decreased by less than 1% within 5 minutes after preparation. All prepared Fe(VI) stock solutions were typically used within 10 minutes after preparation without any significant decomposition. However, this standardization technique was not able to accurately determine the Fe(VI) concentration below 10μM, which required the use of appropriate dilution factors to obtain the desired concentration. The working solutions of Fe(VI) were prepared by diluting the stock solution with a 5mM Na_2HPO_4 /1mM $Na_2B_4O_7$ buffer, which made them more stable than the stock solution, with a decrease of less than 1% within 10 minutes.

Text S2 Analytical Methods.

The formation H_2O_2 were determined after the reaction of DPD with Fe(VI) in the 300mM phosphate buffer (pH=6). H_2O_2 was quantified by the horseradish peroxidase (HRP)-catalyzed oxidation of DPD to $DPD^{\bullet+}$ by H_2O_2 (H_2O_2 + 2DPD → $2DPD^{\bullet+}$, the HRP-DPD method). Use of the HRP/DPD method to determine H_2O_2 formation from the Fe(VI)-DPD reaction is advantageous because the system already contains residual DPD after the completion of the ferrate(VI)-DPD reaction. Accordingly, 50 μL of the HRP stock solution (1 mg mL^{-1}) was added into 5 mL of a solution where the Fe(VI)-DPD reaction was completed. The increase of the absorbance at 551 nm (ΔA551) was measured 1 min after the addition of HRP.

The formation final iron oxidation state (Fe(III) vs. Fe(II)) were determined after the reaction of DPD with ferrate(VI). 0.5 mL of a bipyridine (BPY) stock solution (5 mM) was added to the reaction solution (5 mL). BPY forms a complex with Fe(II) that has a maximum absorption at 552 nm (ε = 8650 M^{-1} cm^{-1}) [1]. The absorbance change at 510 nm indicating that Fe(III) not Fe(II) is the final product from the Fe(VI)-DPD reaction.

Text S3 Experimental procedure for the stoichiometric coefficient between the reaction Fe(VI) of DPD.

Experiments were conducted by adding a constant amount (1.0 mL) of Fe(VI) stock solution (250.0 μM) into varied concentrations of DPD solutions (49.0 mL), where the range of $[DPD]_0$ / $[Fe\ (VI)]_0$ is 0.1~500. Formation of $DPD^{\bullet+}$ was monitored at 551 nm and consumption of DPD was determined at 240 nm with UV-visible spectrophotometry (UV-9000, SHANGHAI METASH INSTRUMENTS CO., LTD, China).

Text S4 Experimental procedure for the determination of the reaction rate constant between Fe(VI) and phenol.

The rate constant of the reaction between Fe(VI) (15 μM) and phenol was determined under pseudo first-order conditions in excess of phenol (0.15 mM) by using a dispenser system (DispensMate, Dragon Laboratory Instruments Limited. China) and an UV-Vis spectrophotometer. 2 mL of sample solution was withdrawn with a dispenser at specific time intervals into a 3 mL of solution containing phosphate buffer and DPD reagent where the remaining Fe(VI) leads to a coloration of the solution. After 30 s, the solution was transferred into a 1 cm quartz cell to measure the absorbance at 551 nm. The residual concentration of Fe(VI) was then calculated.

Text S5 Experimental procedure for investigate the oxidative capacity of ferrate under buffer solutions.

The formation of $DPD^{\bullet+}$ was studied in 300mM carbonate, 300mM borate, 300mM phosphate and 30mM pyrophosphate buffers, respectively. All solutions were at pH 7.2. Experiments were performed by adding different concentrations of Fe(VI) working solutions (2 mL) to constant concentration DPD solutions (3 mL). The DPD solutions (5 mM) were prepared in the above different types of buffer solutions with NaOH and H_2SO_4 to make sure the pH maintained at 7.2. The Fe(VI) solutions (120 μM) were prepared in 1 mM $Na_2B_4O_7$ buffer (pH=9.2), then Fe(VI) working solution (15~120 μM) was obtained by diluting. Formation of $DPD^{\bullet+}$ was monitored at 551 nm.

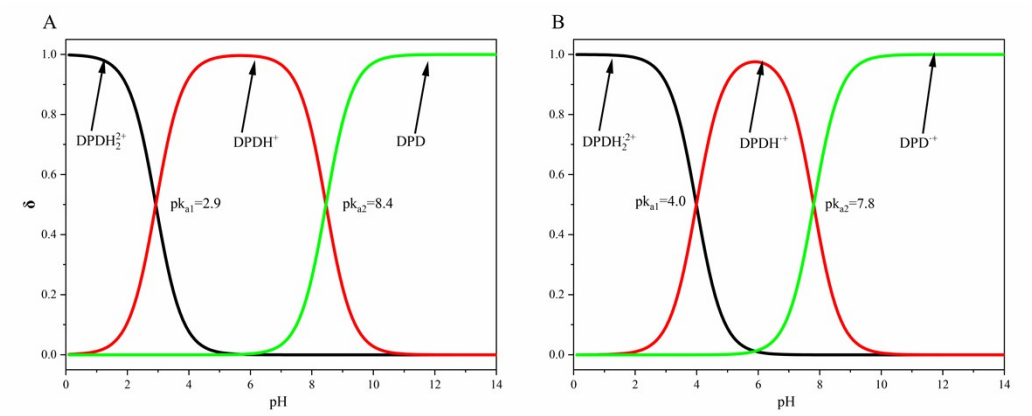


Fig. S1 The morphological distribution of the DPD (A) and DPD$^{\bullet+}$ (B) species at different pH. [2]

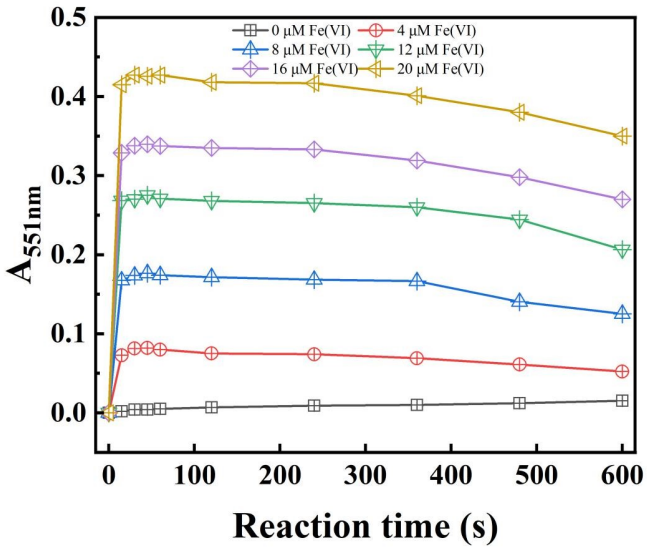


Fig. S2 Effect of reaction time on the absorbance of the formed $DPD^{\bullet+}$ measured at 551 nm within 10 min.

Experimental conditions: $[Fe(VI)]_0$= 0~20 μM, pH = 6 (300 mM phosphate buffer), $[DPD]_0$ = 15 mM, reaction time = 10 min.

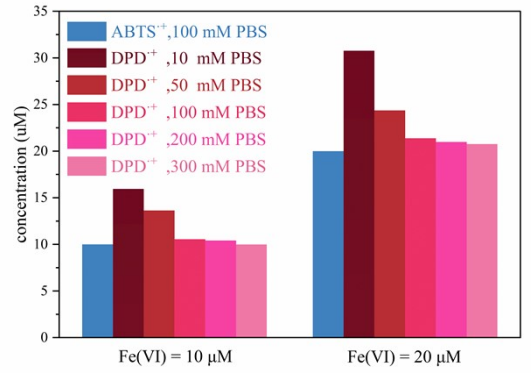


Fig. S3 Effect of buffer concentration solution for $DPD^{\bullet+}$ generated after the oxidation of DPD with Fe(VI).

Experimental conditions: pH =6, $[DPD]_0$ = 5 mM, $[Fe(VI)]_0$ = 0 ~ 20 μM, reaction time =30 s. Where, $[ABTS]_0$=30 μM, 100mM phosphate buffer (pH = 7).

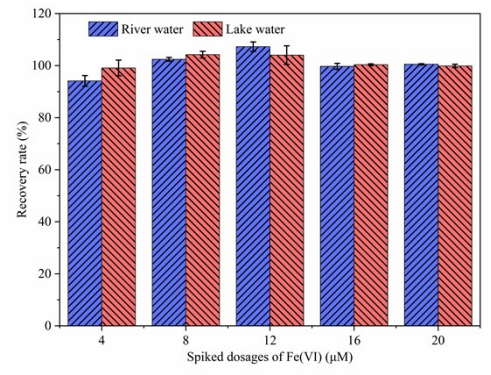


Fig. S4 Recovery test of DPD method with various spiked dosages of Fe(VI).

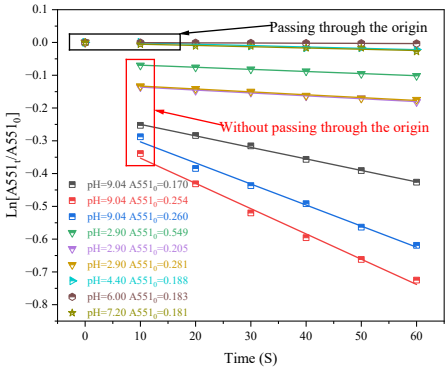


Fig. S5 Stability of DPD at different pH values.

Experimental conditions: pH =2.90、4.40、6.00、7.20、9.04 (100 mM phosphate buffer).

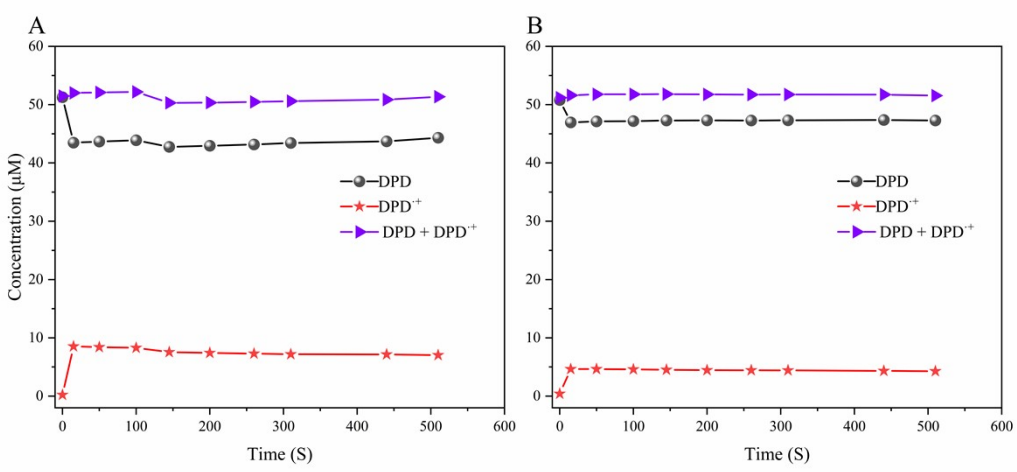


Fig. S6 Evolution of DPD, $DPD^{\bullet+}$ and DPD + $DPD^{\bullet+}$ in the reaction of DPD with Fe(VI) at PH 6.

Experimental conditions: $[DPD]_0$ = 50 μM, $[Fe\ (VI)]_0$ = 20 for (A),10 for (B) μM, pH = 6 (200 mM phosphate buffer).

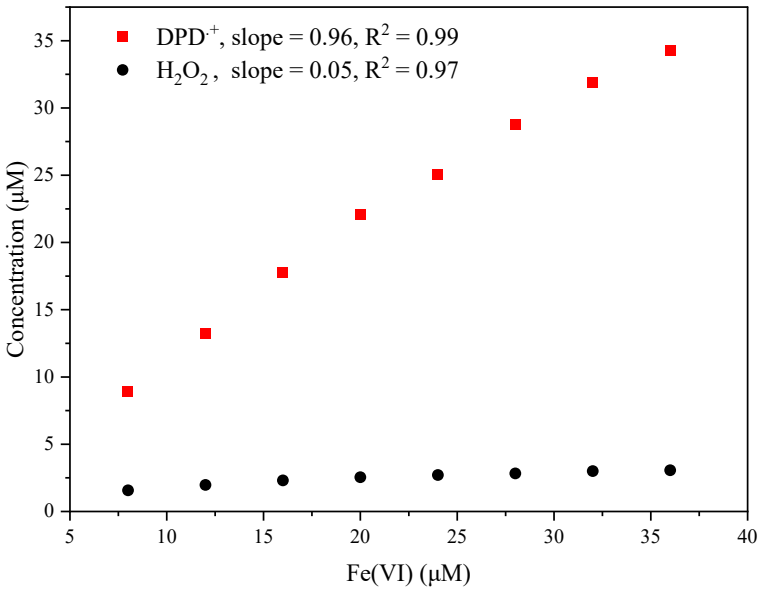


Fig. S7 Formation of $DPD^{\bullet+}$ and H_2O_2 were measured for each Fe(VI) dose after complete consumption of Fe(VI).

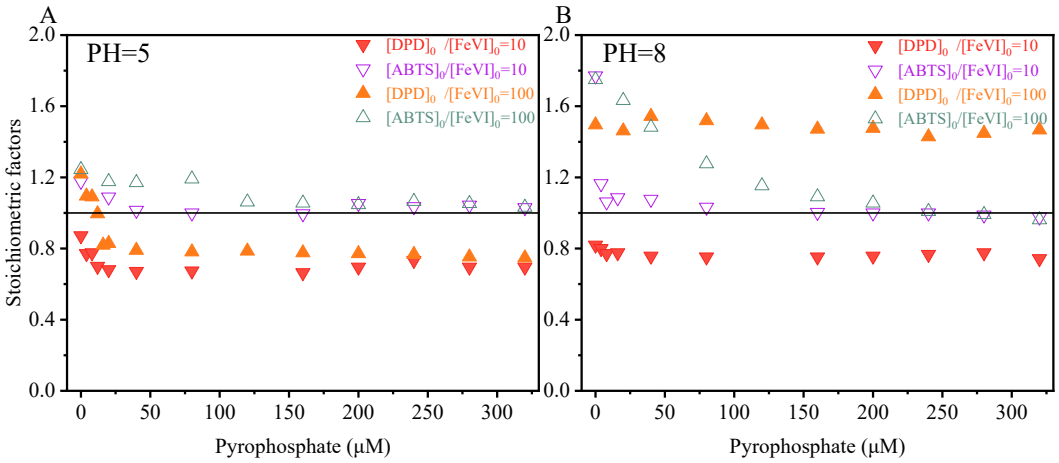


Fig. S8 Influence of pyrophosphate on the production of $DPD^{\bullet+}$ /$ABTS^{\bullet+}$at DPD/ABTS oxidized by Fe(VI).

Experimental conditions: $[Fe(VI)]_0$ = 10 μM, $[DPD]_0$ = 0.1 or 1 mM,[pyrophosphate]$_0$= 0~360 μM,pH = (A) for 5 (300mM acetate buffer) and (B) for 8 (10mM borate buffer).

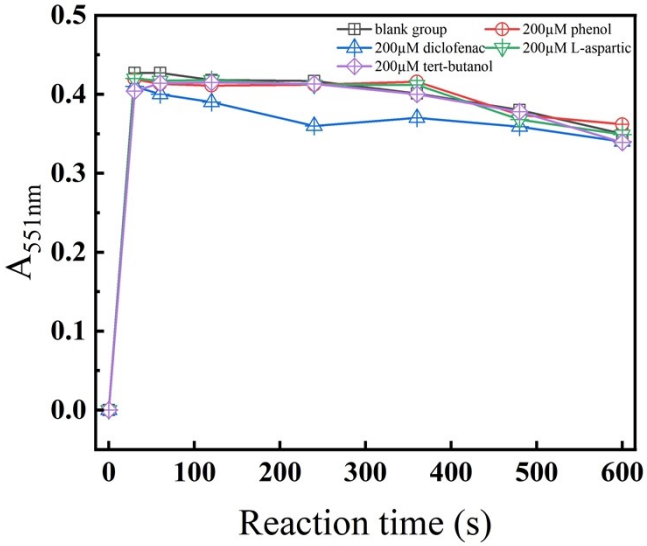


Fig. S9 Effect of phenol, diclofenac, L-aspartic acid, and tert-butanol on the absorbance of the formed $DPD^{\bullet+}$ measured at 551 nm.

Experimental conditions: $[Fe(VI)]_0$= 20 μM, pH = 6 (300 mM phosphate buffer), $[DPD]_0$ = 15 mM, reaction time = 10 min, $[phenol]_0$= $[diclofenac]_0$= $[L\text{-aspartic acid}]_0$= $[tert\text{-butanol}]_0$= 200 μM.

Table S1 Chemical properties and structures.

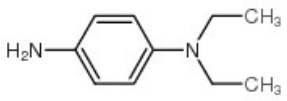
	Formula	Mol. Weight	pKa	Structure
N,N-Diethyl-p-phenylenediamine (DPD)	$C_{10}H_{16}N_2$	164.24	pKa_1=2.9 pKa_2=8.4	

Table S2 Water quality parameters of Pearl river water and Lake water.

Water parameters	pH	UV254	DOC (mg C/L)	Alkalinity (mg/L)
River water	6.5	0.107	3.49	5.61
Lake water	7.8	0.176	4.7	3.59

Table S3 Original data of calibration curves in natural water.

[Fe(VI)]/μM		0	4	8	12	16	20	Slope	R^2
River water at 551 nm	Trial1	0	0.078	0.170	0.276	0.338	0.417	0.0212	0.9956
	Trial2	0	0.075	0.167	0.260	0.332	0.428	0.0214	0.9970
	Trial3	0	0.078	0.169	0.264	0.327	0.415	0.0208	0.9991
	Trial4	0	0.074	0.170	0.265	0.327	0.403	0.0205	0.9992
	Trial5	0	0.079	0.170	0.266	0.328	0.416	0.0209	0.9992
	Trial6	0	0.079	0.171	0.266	0.328	0.432	0.0215	0.9991
	Average	0	0.077	0.169	0.267	0.332	0.420	0.0212	0.9990
	Standard Deviation	0	0.0021	0.0012	0.0049	0.0038	0.0093	0.0004	

[Fe(VI)]/μM		0	4	8	12	16	20	Slope	R^2
Lake water at 551 nm	Trial1	0	0.084	0.176	0.274	0.339	0.423	0.0213	0.9975
	Trial2	0	0.080	0.172	0.270	0.336	0.438	0.0218	0.9986
	Trial3	0	0.080	0.173	0.267	0.338	0.420	0.0212	0.9987
	Trial4	0	0.082	0.177	0.264	0.336	0.445	0.0220	0.9971
	Trial5	0	0.082	0.178	0.266	0.336	0.421	0.0211	0.9977
	Trial6	0	0.088	0.176	0.269	0.337	0.446	0.0219	0.9977
	Average	0	0.081	0.174	0.270	0.338	0.427	0.0214	0.9983
	Standard Deviation	0	0.0028	0.0022	0.0032	0.0012	0.0112	0.0004	

Table S4 Original data of LOD and LOQ calculation.

The LOD and LOQ are expressed by the following equations [3]:

$$LOD = \frac{3\sigma}{K_s} \quad \text{(1)}$$

$$LOQ = \frac{10\sigma}{k_s} \quad \text{(2)}$$

where σ is the standard deviation (SD) of the absorbance values obtained from 20 blank samples at the detected wavelength; K_s indicates the slope of the calibration curves.

Item	Ultrapure water at 551 nm	Ultrapure water at 510 nm
Absorbance of blank samples	0.013	0.004
	0.008	0.007
	0.008	0.014
	0.007	0.012
	0.008	0.014
	0.003	0.014
	0.005	0.010
	0.012	0.005
	0.008	0.012
	0.009	0.013
	0.014	0.004
	0.007	0.015
	0.013	0.014
	0.014	0.016
	0.014	0.006
	0.006	0.016
	0.008	0.014
	0.009	0.014
	0.012	0.016
	0.006	0.014
	0.011	0.011
Standard deviation (σ)	0.0032	0.0040
Slope of calibration curve (k_s) ($M^{-1}cm^{-1}$)	20700	19900
LOD (μM)	0.47	0.61
LOQ (μM)	1.57	2.03

Table S5 Original data of the recovery test for the determination of Fe(VI) using DPD method in real water samples.

Sample Name		River water at 551 nm				
Fe(VI)/ μM		4.00	8.00	12.00	16.00	20.00
Detected concentration (μM)	Trial 1	3.77	8.22	13.33	16.30	20.13
	Trial 2	3.62	8.08	12.54	16.03	20.18
	Trial 3	3.76	8.18	12.77	15.79	20.04
	Trial 4	3.80	8.21	12.82	15.81	20.06
	Trial 5	3.82	8.24	12.86	15.83	20.07
	Trial 6	3.86	8.27	12.89	15.87	20.11
	Trial 7	3.72	8.16	12.88	16.04	20.12
Average detected concentration (μM)		3.76	8.19	12.87	15.95	20.10
Standard deviation		0.08	0.06	0.24	0.18	0.05
Average recovery rate		94.10 %	102.42 %	107.26 %	99.71 %	100.50 %
relative standard deviation		2.04%	0.75%	1.83%	1.16%	0.24%

Sample Name		Lake water at 551 nm				
Fe(VI)/ μM		4.00	8.00	12.00	16.00	20.00
Detected concentration (μM)	Trial 1	4.00	8.38	12.03	16.15	20.16
	Trial 2	4.00	8.19	12.67	16.00	19.95
	Trial 3	3.81	8.24	12.57	16.10	20.00
	Trial 4	3.90	8.43	12.81	16.00	19.76
	Trial 5	3.90	8.48	13.05	16.00	20.05
	Trial 6	4.19	8.38	12.48	16.05	19.86
	Trial 7	3.94	8.27	11.76	16.08	20.04
Average detected concentration (μM)		3.96	8.34	12.48	16.05	19.97
Standard deviation		0.12	0.11	0.45	0.06	0.13
Average recovery rate		99.08 %	104.21 %	104.00 %	100.33 %	99.87 %
relative standard deviation		3.01%	1.27%	3.58%	0.36%	0.66%

Table S6 The recovery test of DPD method and ABTS method for Fe(VI) determination with different interference ions.

Sample Name		DPD method			ABTS method		
Fe(VI):Ca(II)	□	1:10	1:50	1:100	1:10	1:50	1:100
Detected concentration (μM)	Trial 1	20.29	18.81	17.62	20.21	18.76	14
	Tria l2	19.62	18.52	15.52	17.29	16.76	15.12
	Trial 3	20.75	19.03	17.39	19.41	18.33	14.64
	Trial 4	20.76	19.10	16.70	18.97	17.61	15.70
	Trial 5	21.19	19.98	17.20	19.66	18.09	15.20
	Trial 6	21.97	19.93	16.96	19.58	18.00	16.10
	Trial 7	21.89	19.97	17.64	19.93	18.41	15.80
Average detected concentration(μM)		20.92	19.33	17.00	19.29	18.00	15.22
Standard deviation		0.84	0.61	0.74	0.97	0.65	0.73
Average recovery rate		104.62	96.67	85.02	96.46	89.98	76.11
relative standard deviation		4.02	3.18	4.34	5.00	3.63	4.79
Fe(VI):Mg(II)	□	1:10	1:50	1:100	1:10	1:50	1:100
Detected concentration (μM)	Trial 1	20.71	20.14	19.57	21.41	20.44	20.18
	Tria l2	20.19	19.14	18.95	20.4	21.58	20.32
	Trial 3	20.72	19.84	19.50	21.34	21.08	20.43
	Trial 4	20.65	19.63	20.20	21.86	21.54	20.88

	Trial 5	21.30	20.12	20.15	22.31	21.97	21.60
	Trial 6	21.61	20.55	20.44	22.19	21.89	21.39
	Trial 7	21.98	20.51	20.38	22.94	22.23	22.39
Average detected concentration(μM)		21.02	19.99	19.88	21.78	21.53	21.03
Standard deviation		0.63	0.50	0.55	0.82	0.61	0.81
Average recovery rate		105.11	99.95	99.41	108.90	107.66	105.14
relative standard deviation		2.98	2.50	2.79	3.77	2.82	3.85
Fe(VI):NH_4^+		1:10	1:50	1:100	1:10	1:50	1:100
Detected concentration (μM)	Trial 1	21.9	19.1	17.29	21.68	20.15	16.03
	Tria l2	19.86	17.9	14.81	20.09	22.03	20.09
	Trial 3	21.67	20.95	20.67	22.5	20.46	18.53
	Trial 4	21.77	19.90	18.22	22.34	21.86	19.04
	Trial 5	21.22	19.99	18.64	22.27	22.41	19.79
	Trial 6	22.30	21.23	19.86	22.41	22.05	19.40
	Trial 7	22.35	21.31	19.57	22.39	22.95	19.57
Average detected concentration(μM)		21.58	20.05	18.44	21.96	21.70	18.92
Standard deviation		0.85	1.25	1.95	0.87	1.02	1.37
Average recovery rate		107.91	100.27	92.19	109.78	108.52	94.61
relative standard		3.95	6.22	10.58	3.95	4.72	7.26

	deviation							

Table S7 The recovery of Fe(VI) determined by DPD method under different interfering ions with concentrations closer to natural water.

Fe(VI):Interfering Ion or Humic Acid Concentration	Recovery of DPD Method (%)
Fe(VI):Ca^{2+}=1:5	98.7±2.1
Fe(VI):NH_4^+=1:1	101.3±1.8
Fe(VI):Na^+=1:5	97.2±2.3
Fe(VI):K^+=1:5	95.8±2.5
Fe(VI):Fe^{3+}=1:0.05	102.5±1.6
Fe(VI):Cu^{2+}=1:0.05	99.1±2
Fe(VI):Cl^-=1:50	96.5±2.4
Fe(VI):SO_4^{2-}=1:50	100.5±1.2
Fe(VI):HCO_3^-=1:50	99.8±1.5
[Humic Acid] =0mg/L	100.2±1.7
[Humic Acid] =1mg/L	99.7±1.5
[Humic Acid] =5mg/L	95.3±2.5

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[2] H.E. Moore, M.J. Garmendia, W.J. Cooper, Kinetics of monochloramine oxidation of N,N-diethyl-p-phenylenediamine, Environmental science & technology, 18 (1984) 348-353.

[3] J. Uhrovčík, Strategy for determination of LOD and LOQ values – Some basic aspects, Talanta, 119 (2014) 178-180.