Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2018

Supporting Information for

A novel and simple spectrophotometric method for detection of nitrite in aqueous

Huihui Wang[a], Nanwei Wan*[a], Lin Ma[a], Zhongqiang Wang[a], Baodong Cui[a], Wenyong Han[a] and Yongzheng Chen*[a]

a Generic Drug Research Center of Guizhou Province, Green Pharmaceuticals Engineering Research Center of Guizhou Province, School of Pharmacy, Zunyi Medical University, Zunyi, 563000, China.

Corresponding Author

* Dr. Nanwei Wan, E-mail: nanweiwan@zmc.edu.cn.

* Prof. Dr. Yongzheng Chen, E-mail: yzchen@zmc.edu.cn.

Table of Contents

1. General methods

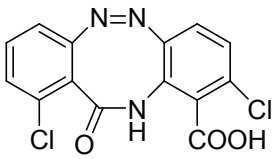
All reagents and solvents were obtained commercial suppliers and were used without further purification. MilliQ water was used throughout. ^{1}H NMR (400 MHz) chemical shifts were reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data were reported as follows: chemical shift, multiplicity (s = singlet, br s= broad singlet, d = doublet, t = triplet), coupling constants (Hz) and integration. ^{13}C NMR (100 MHz) chemical shifts were reported in ppm (δ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard.

2. Spectroscopic procedure

UV-visible absorption spectra were collected over a T9CS spectrophotometer (Beijing Purkinje General Instrument). Unless otherwise stated, all chemicals are of analytical reagent grade and test water is deionized water. A standard nitrite analysis procedure was performed as follows: 25.0 mL ACBA solution in a 50 mL volumetric flask was mixed with 25 mL of standard concentration nitrite solution in the working range (final concentration of 2-50 □M $NaNO_2$). The mixture was incubated in water bath at a given temperature under illuminating with a UV lamp (λ=340 nm, 20W) until to the analytical time. The absorbance at 435 nm of a 1.00 cm quartz cell was measured while using a blank reagent prepared in the same manner but without nitrite as a reference.

3. Purification and characterization of the cyclic azobenzene product 7

In order to purify the chromogenic azoic compound, 2.5 L (10×250 mL) reaction mixture of 2 mM ACBA against 50 μM $NaNO_2$ was extracted with ethyl acetate. The organic phase was separated, combined, and dried on anhydrous sodium sulfate. And then the organic phase was concentrated by distillation under reduced pressure. 21 mg of a yellow powdery solid was obtained by recrystallization and filtration. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.30 (s, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.50 (t, J = 8.2 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.21 (d, J = 9.6 Hz, 1H), 6.59 (d, J = 9.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.33 (s), 169.86 (s), 166.38 (s), 162.67 (s), 141.09 (s), 138.25 (s), 132.47 (s), 132.20 (s), 131.69 (s), 126.59 (s), 122.73 (s), 117.88 (s), 114.87 (s), 97.27 (s). EI-HRMS calculated for $C_{14}H_7Cl_2N_3O_3$ $[M + H]^+$, 335.9937; found, 336.038.



(*Z*)-1,9-dichloro-12-oxo-11,12-dihydrodibenzo[c,g][1,2,5]triazocine-10-carboxylic acid

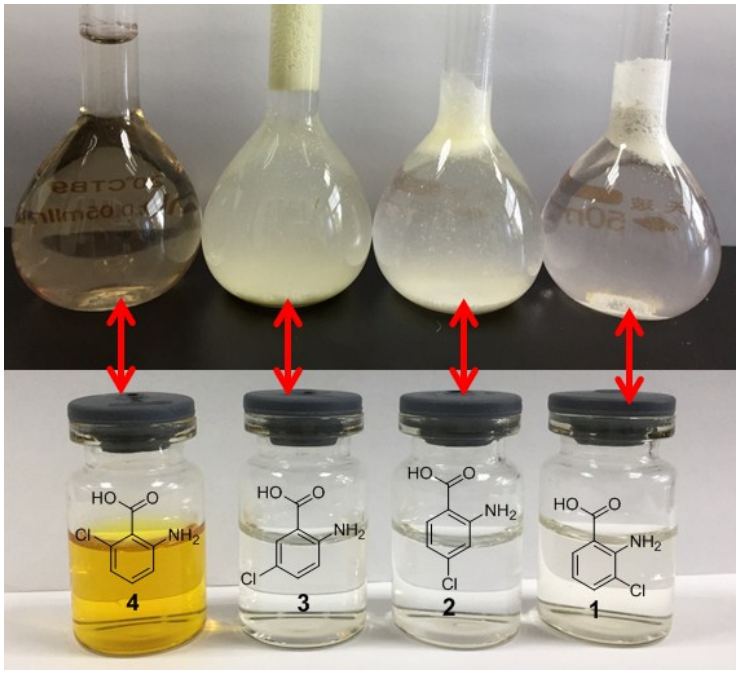


Fig. S1 The solubility result (above) of the four chlorinated 2-aminobenzoic acid compounds (20 mM) and their coloration selectivity (below) toward nitrite (3 mM).

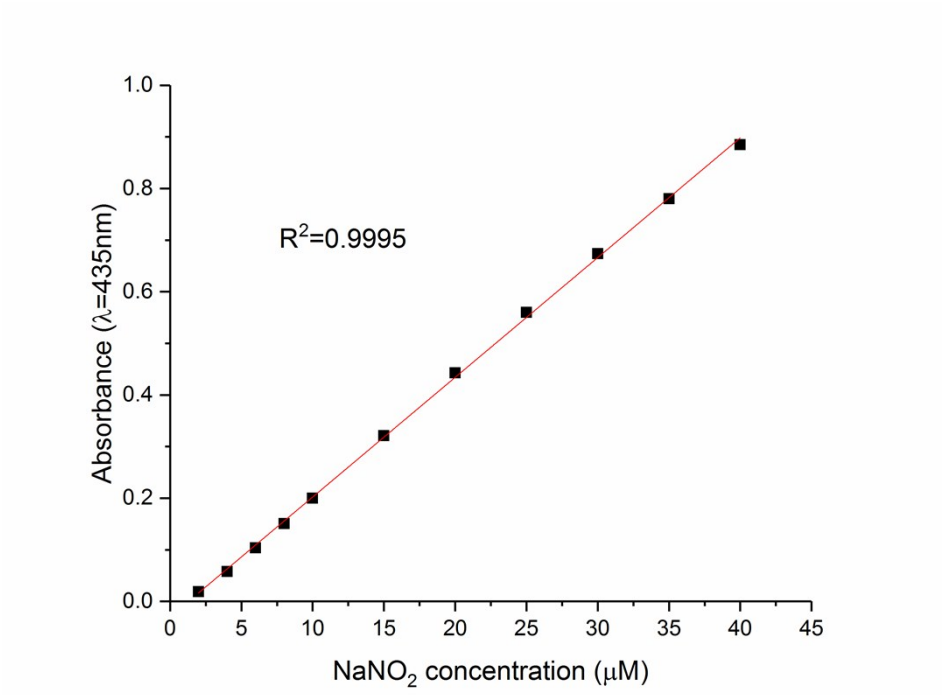


Fig. S2 The calibration curve toward 2-40 μM $NaNO_2$ determined with 2 mM ACBA at λ=435 nm. ACBA were prepared in deionized water.

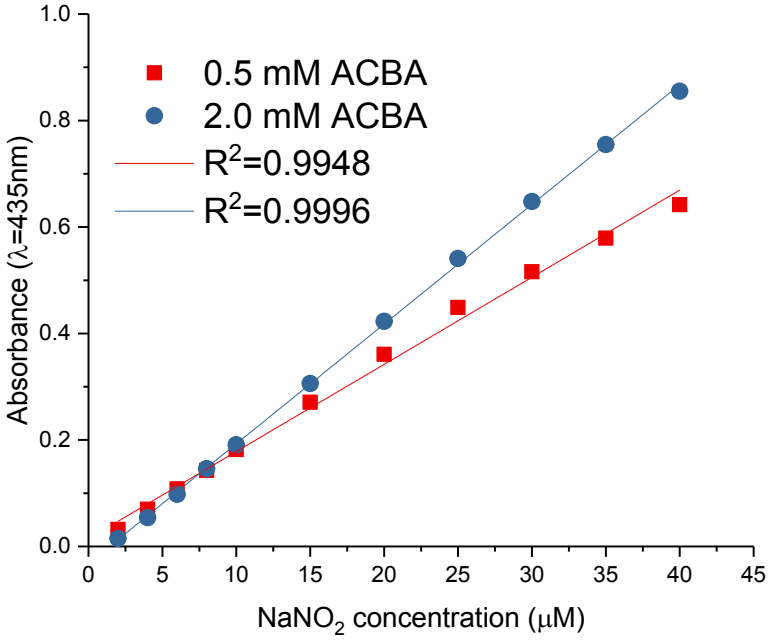


Fig. S3 The calibration curve toward 2-40 μM $NaNO_2$ determined with 0.5 mM and 2 mM ACBA at λ=435 nm. ACBA were prepared in 0.7 M acetic acid solution.

Table S1. Investigation of the accuracy of the ACBA method.[a]

Entry	ACBA mehtod[b]			Griess method[c]		
	Abs[d]	Conc.[e] (μM)	Recovery (%)	Abs[d]	Conc.[e] (μM)	Recovery (%)
1	0.242	12.53	100.54	0.574	12.55	100.66
2	0.240	12.44	100.00	0.572	12.51	100.31
3	0.242	12.53	100.54	0.571	12.49	100.14
4	0.239	12.40	99.42	0.572	12.51	100.31
5	0.238	12.35	99.05	0.572	12.51	100.31
6	0.236	12.26	98.31	0.573	12.53	100.48
Avg. Recovery (%)			99.61			100.37
R.S.D. (%)			0.874			0.181

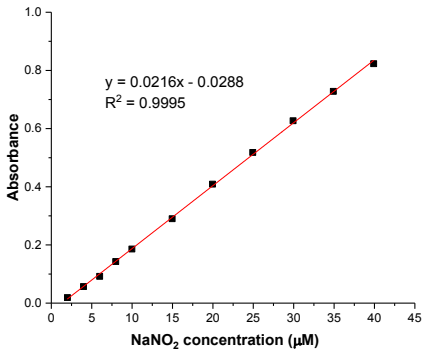
[a] The six samples containing 12.47 μM nitrite were used to test the accuracy of the ACBA method (2 mM ACBA in 0.7 M acetic acid solution) by comparing with the Griess method.

[b] The calibration curve of the ACBA method was $y = 0.0216x - 0.0288$, $R^2 = 0.9995$.

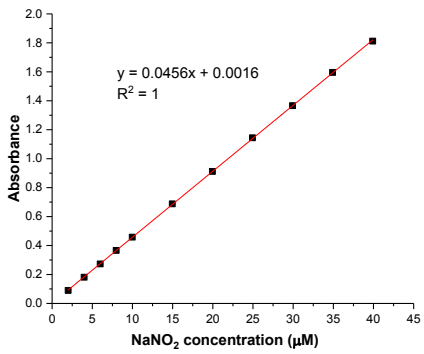
[c] The calibration curve of the Griess method was $y = 0.0456x + 0.0016$, $R^2 = 1$.

[d] The absorbance values determined by the ACBA method or the Griess method.

[e] The nitrite concentration determined by the ACBA method or the Griess method.



The calibration curve of the ACBA method



The calibration curve of the Griess method

Table S2. Investigation of the repeatability of the ACBA method.[a]

Entry	1	2	3	4	5	6
Abs[b]	0.242	0.242	0.242	0.242	0.242	0.241
Conc.[c]	12.54	12.54	12.54	12.54	12.54	12.49
R.S.D. (%)				0.151		

[a] The sample containing 12.47 μM nitrite was used to test the repeatability of the ACBA method (2 mM ACBA in 0.7 M acetic acid solution) by determining six times. The calibration curve was $y = 0.0216x - 0.0288$, $R^2 = 0.9995$.

[d] The absorbance value determined by the ACBA method.

[c] The nitrite concentration determined by the ACBA method.

Table S3. Investigation of the stability of the ACBA method under working conditions.[a]

Time (h)	Calibration curve	Conc. (μM) /(Abs) Sample 1[b]	Recovery (%) Sample 1[c]	Conc. (μM) /(Abs) Sample 2[d]	Recovery (%) Sample 2[e]
0	y = 0.0208x - 0.029 R^2 = 0.9993	17.64/(0.338)	100.8	23.03/(0.450)	102.3
1	y = 0.0207x - 0.0225 R^2 = 0.9984	17.60/(0.342)	100.5	22.97/(0.453)	102.1
2	y = 0.0207x - 0.0212 R^2 = 0.998	17.54/(0.343)	100.2	22.96/(0.454)	102.0
3	y = 0.0204x - 0.0186 R^2 = 0.9978	17.68/(0.342)	101.0	23.07/(0.452)	102.5
4	y = 0.02x - 0.0117 R^2 = 0.9961	17.59/(0.34)	100.5	23.04/(0.449)	102.4
5	y = 0.0195x - 0.0035 R^2 = 0.9944	17.62/(0.34)	100.7	23.15/(0.448)	102.8
6	y = 0.019x + 0.0027 R^2 = 0.9926	17.80/(0.341)	101.7	23.49/(0.449)	104.4

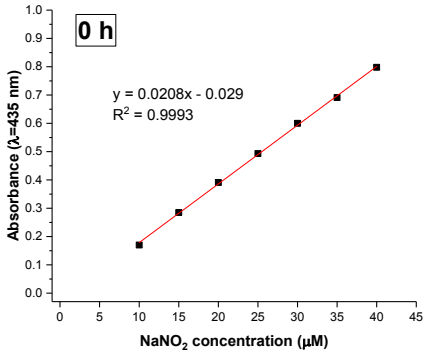
[a] The stability of the ACBA method (2 mM ACBA in 0.7 M acetic acid solution) was tested under working conditions. After reaction for 100 min, the reaction mixtures continue to be incubated at 20 °C and under UV-light illumination. The calibration curves and the two nitrite solution samples (sample 1: 17.5 μM; sample 2: 22.5 μM) were determined respectively at 0, 1, 2, 3, 4, 5 and 6 h.

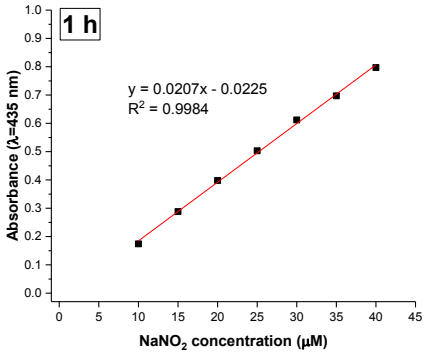
[b] The nitrite concentration/absorbance (λ=435 nm) of sample 1 (17.5 μM nitrite).

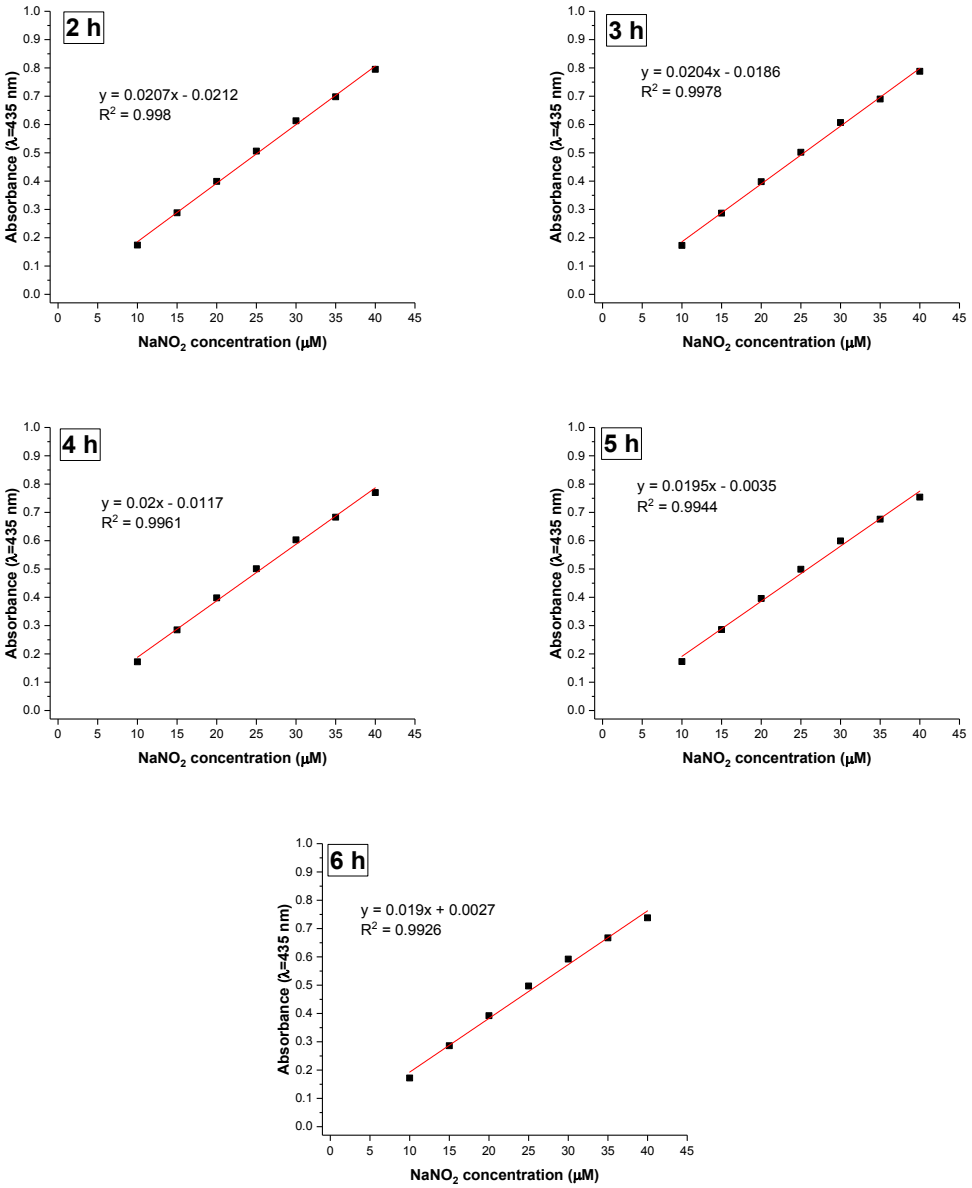
[c] The recovery of sample 1 (17.5 μM nitrite)

[d] The nitrite concentration/absorbance (λ=435 nm) of sample 2 (22.5 μM nitrite).

[e] The recovery of sample 2 (22.5 μM nitrite)







The calibration curves at different times.

Table S4. Investigation of the stability of the ACBA method in normal temperature and normal light conditions.[a]

Time (h)	Calibration curve	Conc. (μM) /(Abs) Sample 1[b]	Recovery (%) Sample 1[c]	Conc. (μM) /(Abs) Sample 2[d]	Recovery (%) Sample 2[e]
0	y=0.0201x - 0.0318 R^2 = 0.999	17.65/(0.323)	100.8	22.78/(0.426)	101.2
1	y = 0.0206x - 0.034 R^2 = 0.9987	17.66/(0.330)	100.9	22.96/(0.439)	102.0
2	y = 0.0205x - 0.0337 R^2 = 0.9982	17.64/(0.328)	100.8	23.00/(0.438)	102.6
3	y = 0.0203x - 0.0306 R^2 = 0.9978	17.71/(0.329)	101.2	23.08/(0.438)	102.6
4	y = 0.0196x - 0.0209 R^2 = 0.9962	17.69/(0.326)	101.1	23.26/(0.435)	103.4
5	y = 0.0189x - 0.0104 R^2 = 0.9936	17.75/(0.327)	101.4	23.57/(0.435)	104.7
6	y = 0.0179x + 0.0052 R^2 = 0.9884	17.86/(0.325)	102.0	23.89/(0.433)	106.2

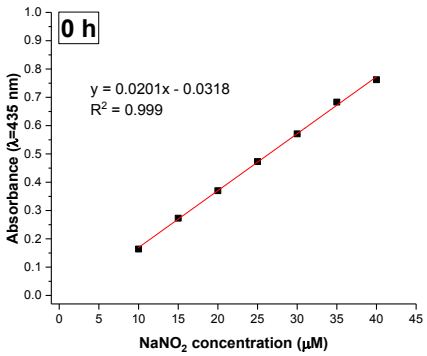
[a] The stability of the ACBA method (2 mM ACBA in 0.7 M acetic acid solution) was tested in normal temperature and normal light conditions. After reaction for 100 min, the reaction mixtures were taken out and put on room temperature and normal light. The calibration curves and the two nitrite solution samples (sample 1: 17.5 μM; sample 2: 22.5 μM) were determined respectively at 0, 1, 2, 3, 4, 5 and 6 h.

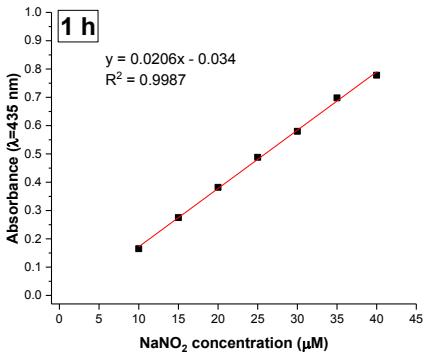
[b] The nitrite concentration/absorbance (λ=435 nm) of sample 1 (17.5 μM nitrite).

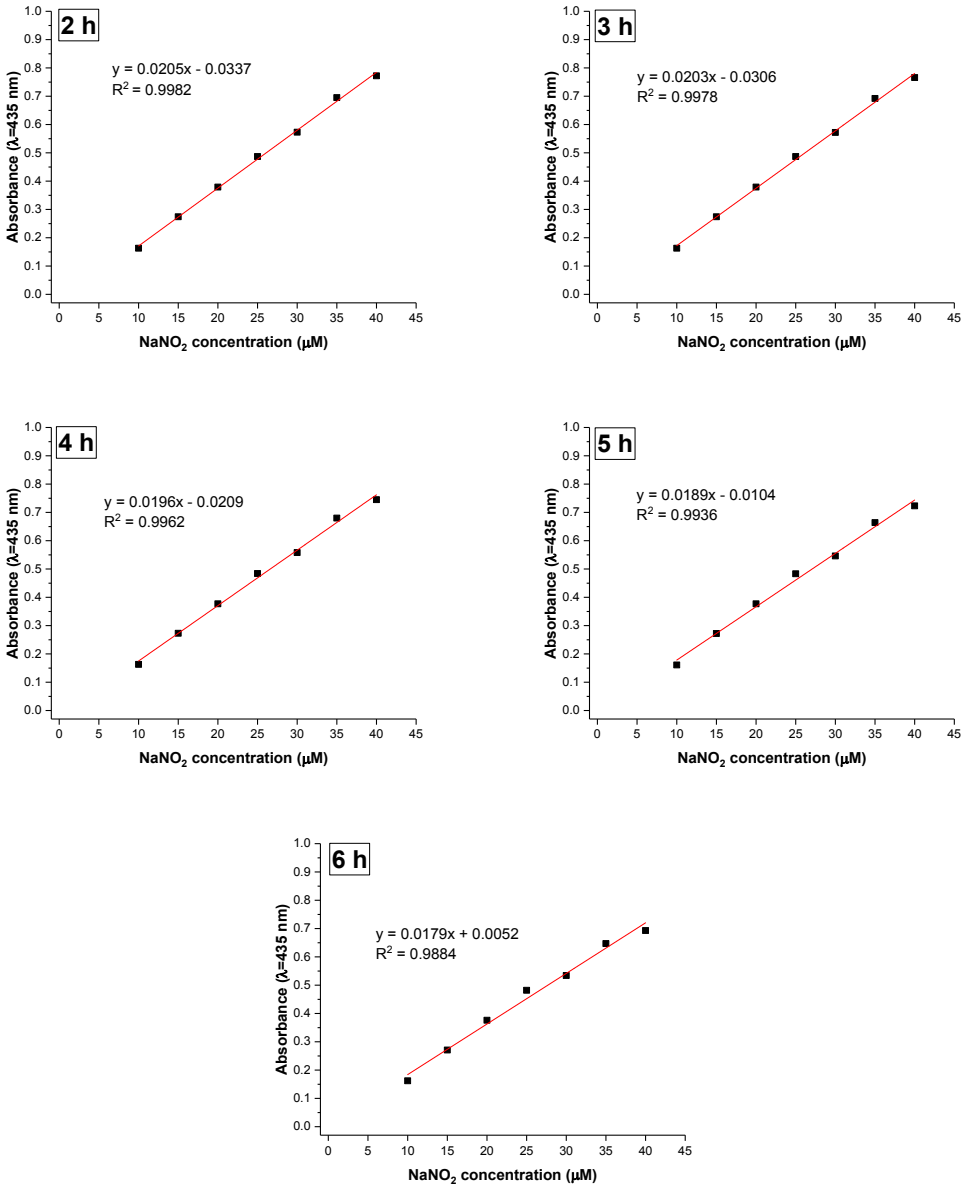
[c] The recovery of sample 1 (17.5 μM nitrite)

[d] The nitrite concentration/absorbance (λ=435 nm) of sample 2 (22.5 μM nitrite).

[e] The recovery of sample 2 (22.5 μM nitrite)







The calibration curves at different times.

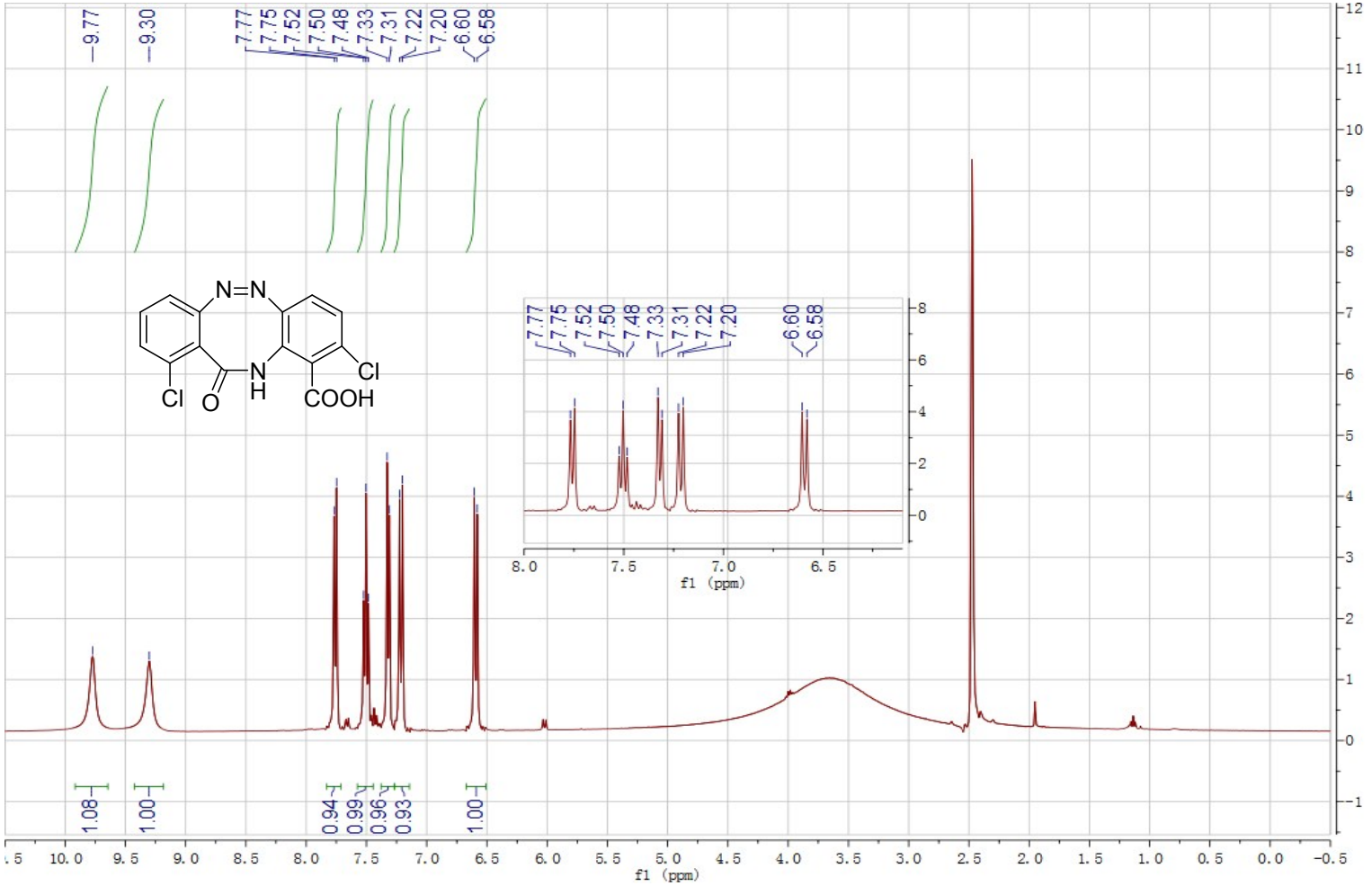


Fig. S4 The ^{1}H NMR spectrum of the cyclic azobenzene product **7**.

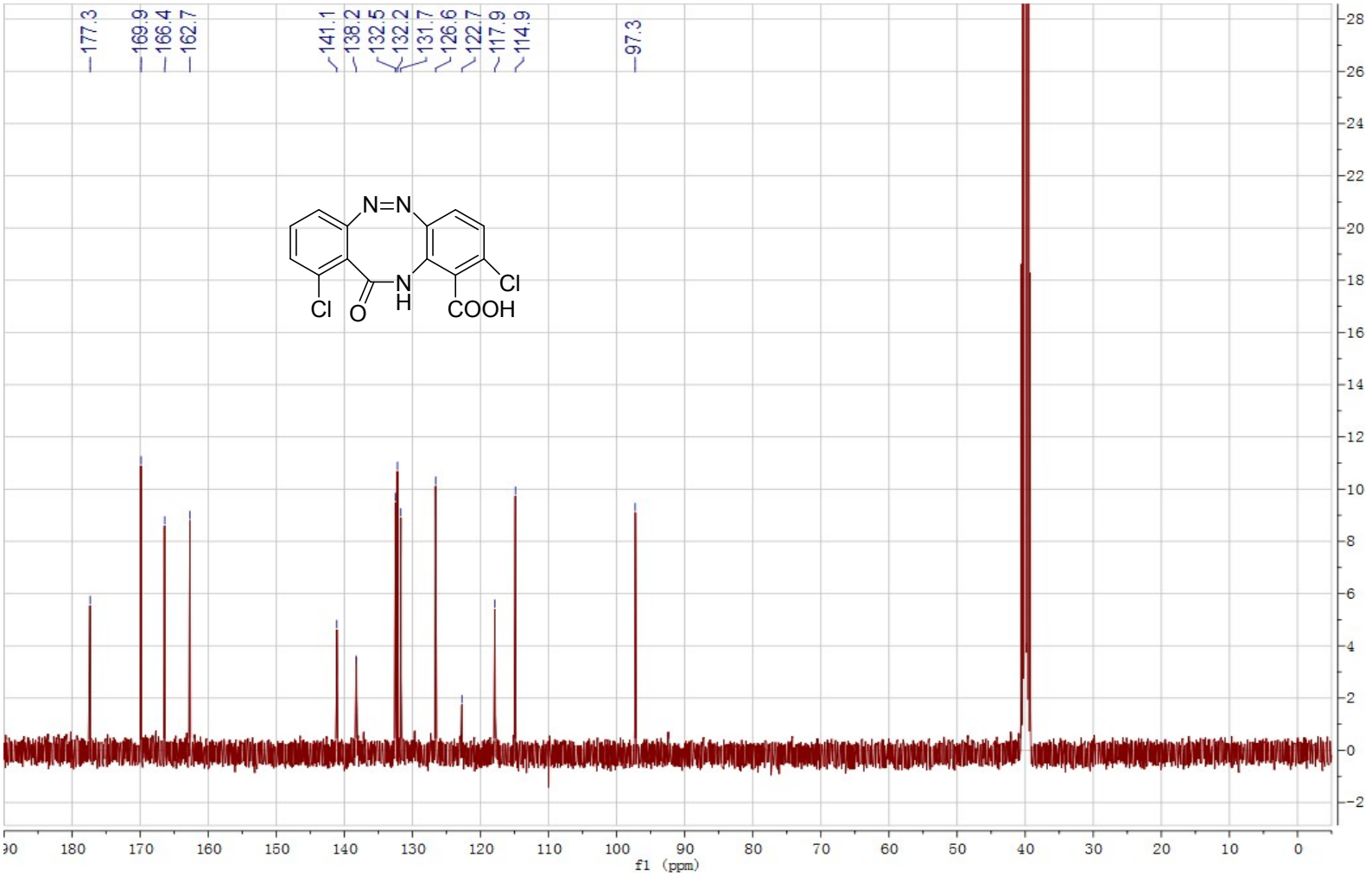


Fig. S5 The ^{13}C NMR spectrum of the cyclic azobenzene product **7**.

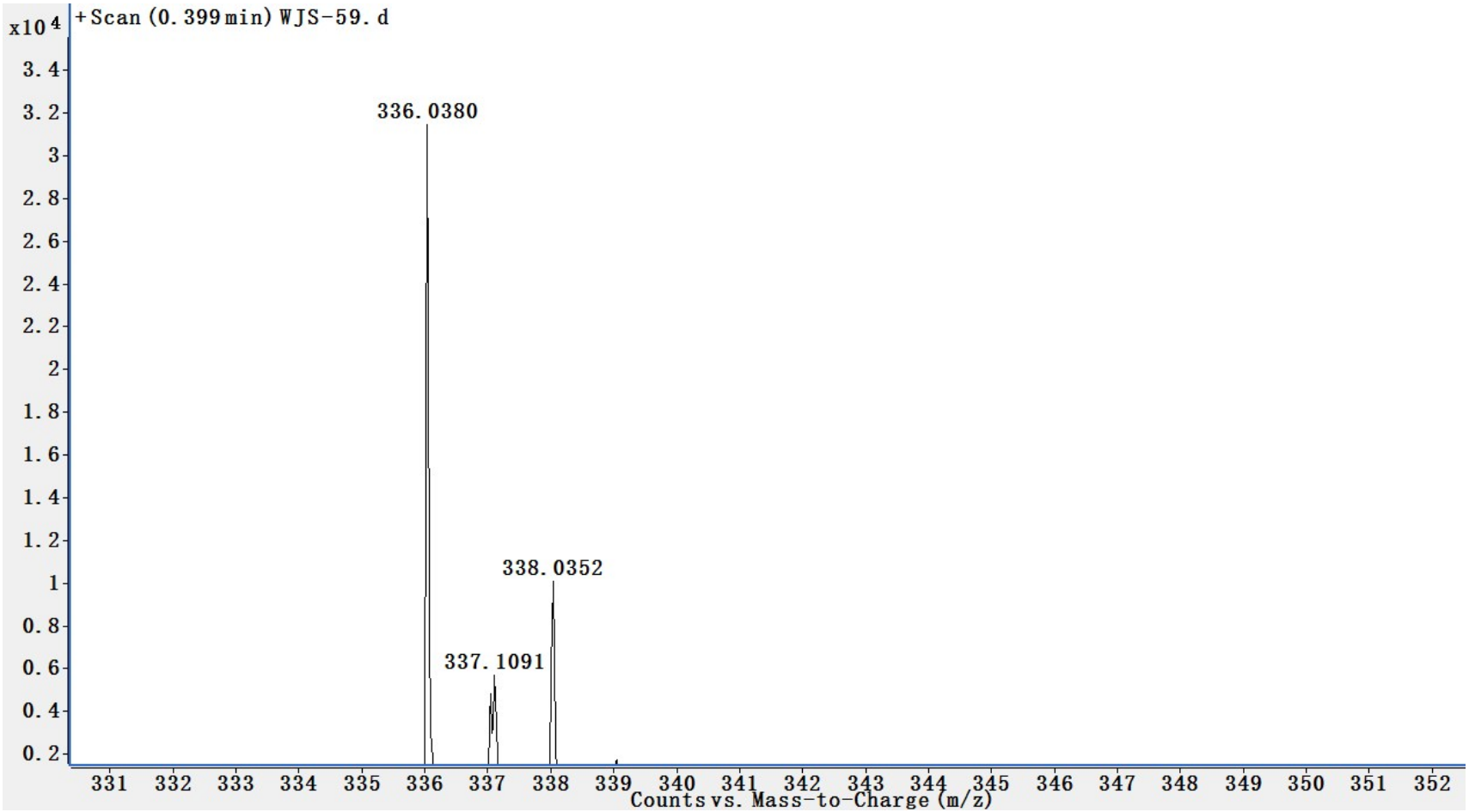


Fig. S6 The HRMS spectrum of the cyclic azobenzene product **7**.