A novel method for artificial antigen synthesis and preparation of a polyclonal antibody for the sensitive determination of leucomalachite green in fish samples by enzyme-linked immunoassay

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Supplemental Material

Synthesis and characterization of the hapten

Various haptens with different spacer arms were synthesized. The specific synthetic scheme was depicted in Figure S1. The NMR and MS spectroscopic results for confirmation of the synthesized LMG hapten I-IV were shown in Figure S2, Figure S3, Figure S4 and Figure S5.

Sensitivities of the antibodies against LMG

Sensitivity comparison of antibodies developed from different haptens. All of the antibodies developed by different haptens were in the same immunological approach. All of the antiserum obtained were optimized and measured according to the steps described in the article. The sensitivity comparison was shown in table S1.

LC-MS/MS procedure

Mobile phases A and B were methanol and water containing 0.1% of formic acid, respectively. The gradient elution program was used as showed in table S2. The injection volume was 5 μL and the flow rate was 0.30 mL/min. The ionization source parameters were listed as follows: capillary voltage, 1.0 kV; cone voltage, 31.0 V; and cone gas flow, 50 L/h. Other MS/MS conditions were used as follows: source temperature, 110℃; desolvation temperature, 400℃; desolvation gas flow, 600 L/h; curtain gas, 15 psi, collision gas, 30 psi. The mass conditions for LMG was optimized during infusion.

The mass spectrum of LMG standard solution (500 μg/L) in scan mode was shown in Figure S 8. Some mass parameters including precursor and product ions, collision energy, dwell time etc. were shown in Table S3. In addition, the chromatogram of the LMG standard solution (10 μg/L) was shown in Figure S9.

Table S1 Sensitivity comparison of the antibodies prepared by hapten I～IV

Type of hapten	Type of the antibody	Antibody dilution	Coating antigen	IC_{50} (ng/mL)
hapten I	antibody I	1:7000	hapten I	2.2
hapten II	antibody II	1:3000	hapten I	22.3
hapten III	antibody III	1:5000	hapten I	8.9
hapten IV	antibody IV	1:5000	hapten I	12.9

Table S2

Gradient elution condition for HPLC

Time (min)	Flow rate (mL/min)	Methanol (%)	Water (0.1% of formic acid, %)
0	0.25	10	90
2	0.25	50	50
7	0.25	90	10
10	0.25	90	10
10.1	0.25	10	90
18	0.25	10	90

Table S3

Multiplereaction monitoring conditions for leucomalachite green.

Compound	Q1 (m/z)	Q3(m/z)	Collision energy (eV)	Dwell time (s)
Isometamidium	331.6	239.3	30	0.3
		315.7	36	0.3
		272.3	40	0.3

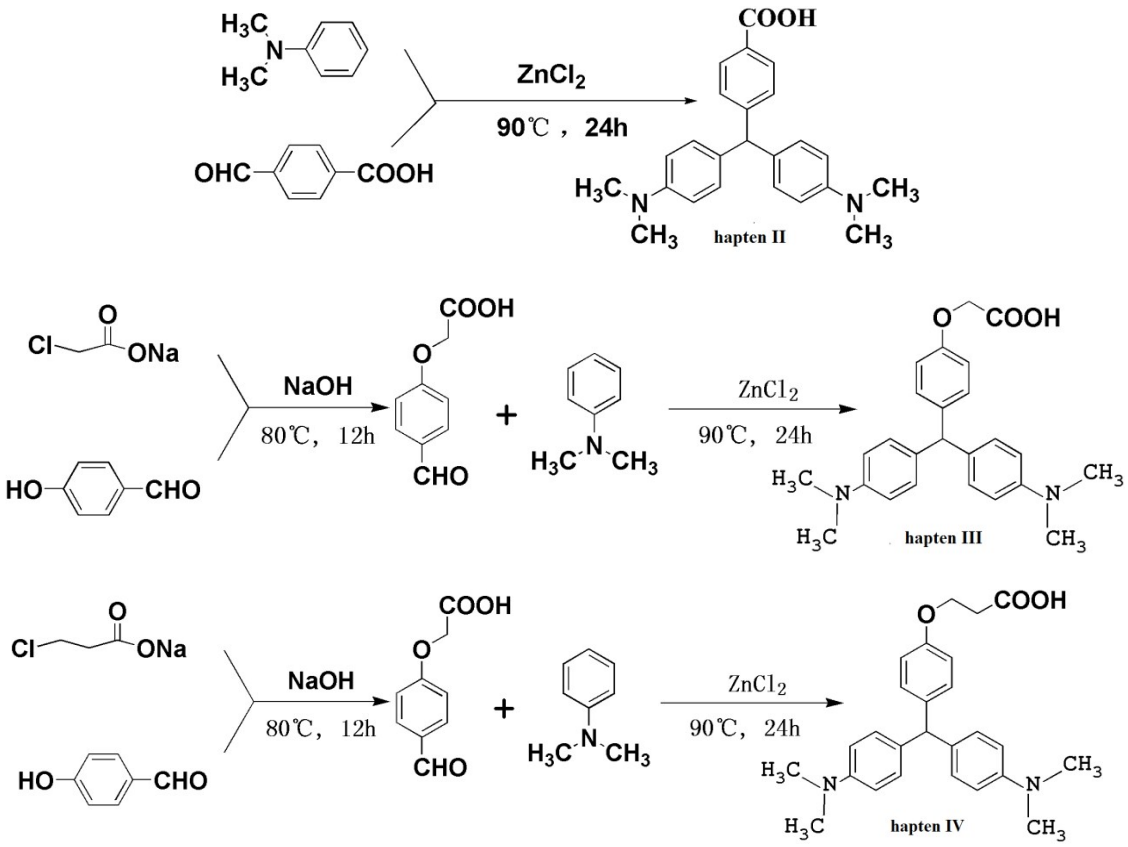

Figure S1. Synthetic scheme for the synthesis of LMG hapten II～IV.

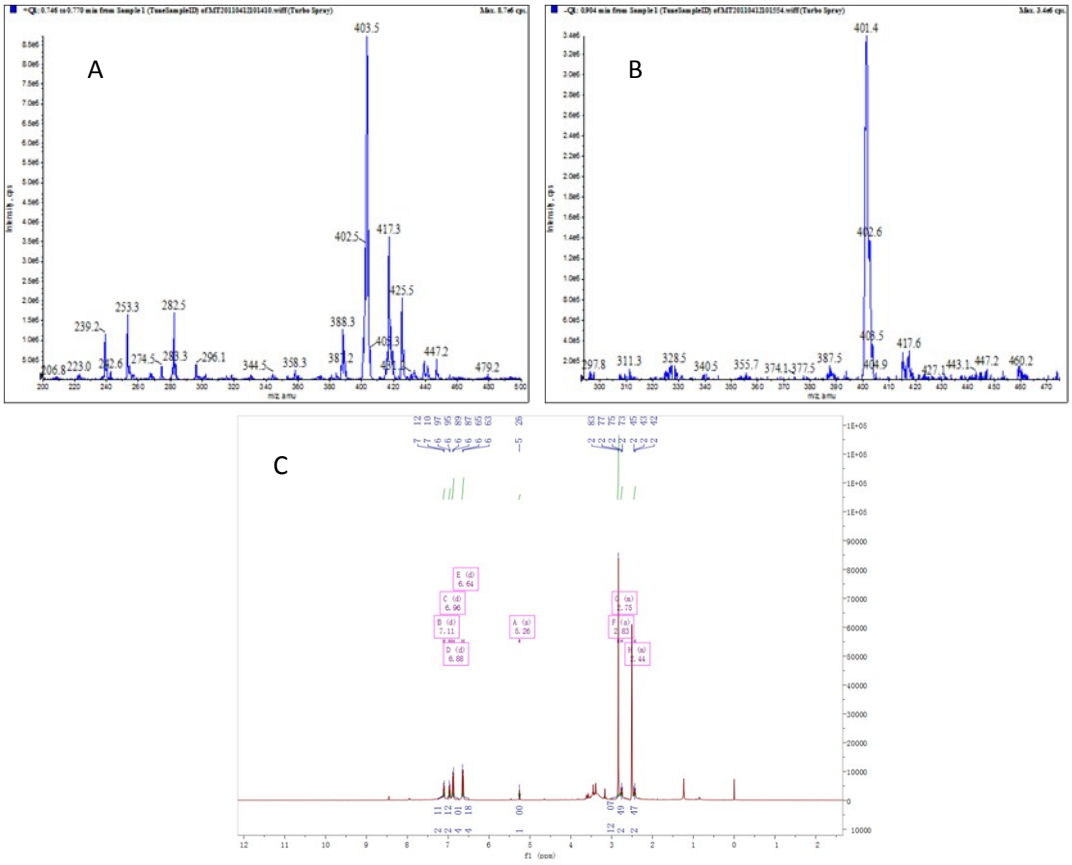

Figure S2. The results of leucomalachite green hapten I (A- The mass spectrogram in positive mode; B- The mass spectrogram in negative mode; C-The NMR spectrum).

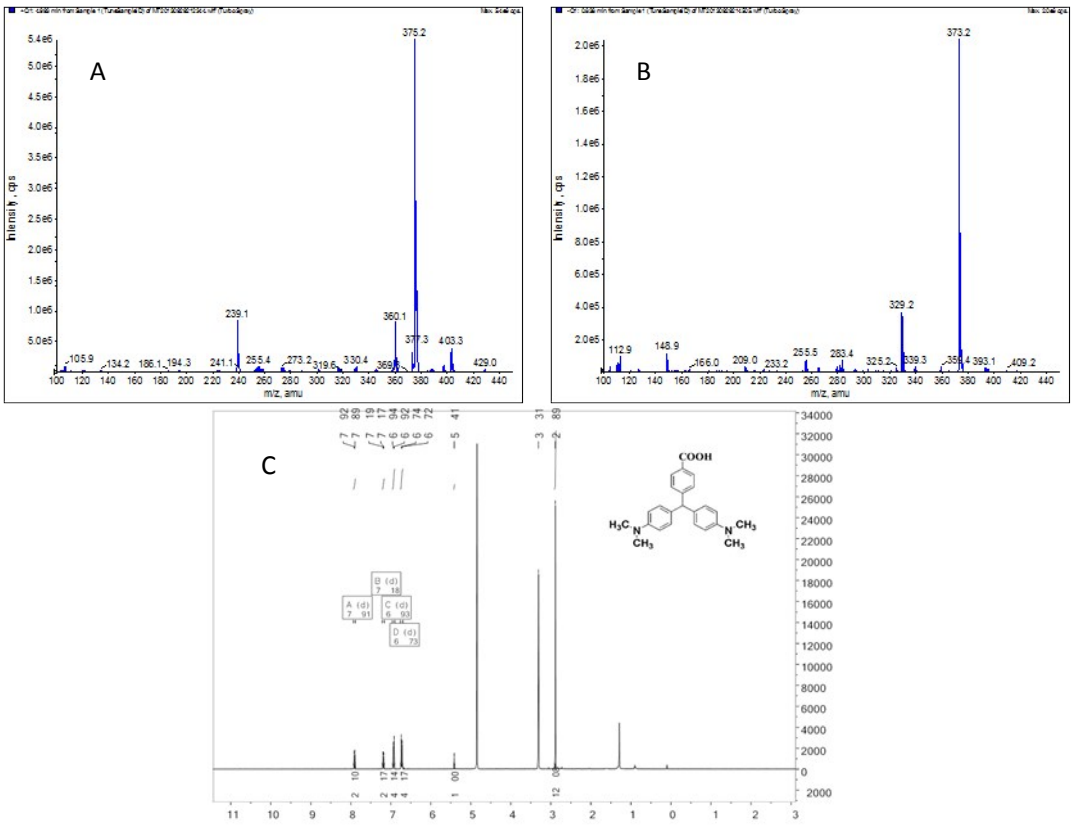

Figure S3. The results of leucomalachite green hapten II (A- The mass spectrogram in positive mode; B- The mass spectrogram in negative mode; C-The NMR spectrum).

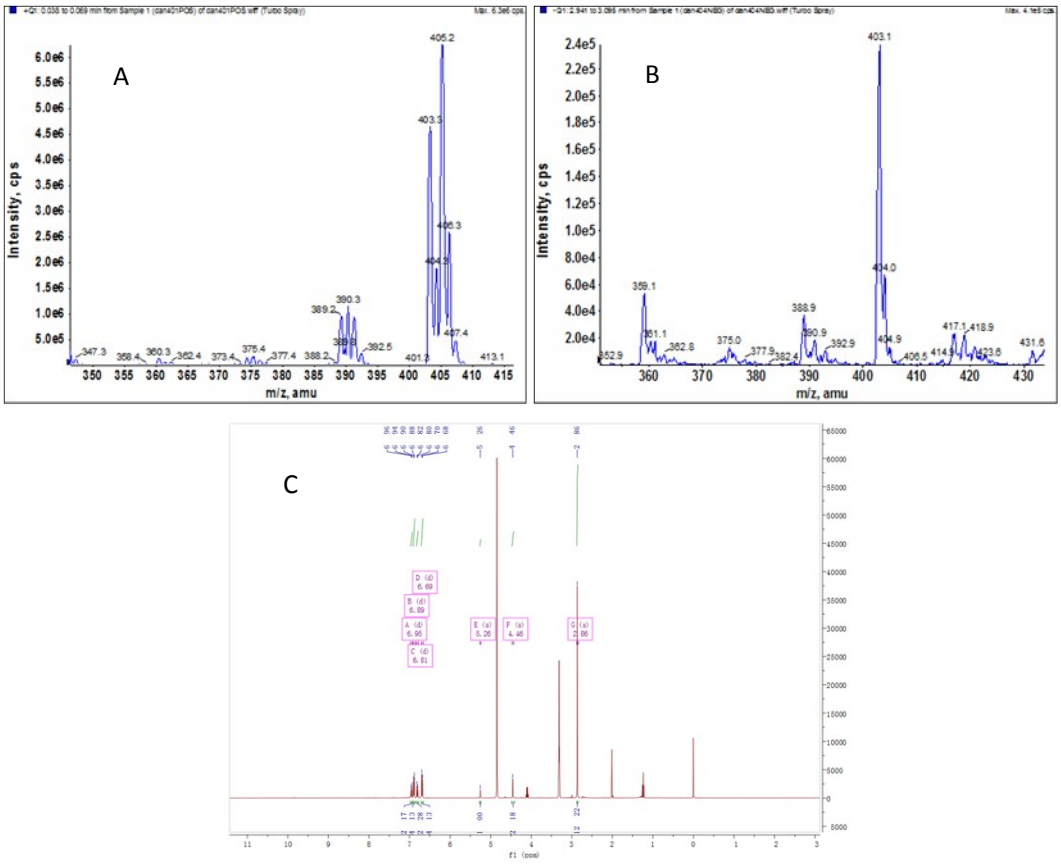

Figure S4. The results of leucomalachite green hapten III (A- The mass spectrogram in positive mode; B- The mass spectrogram in negative mode; C-The NMR spectrum).

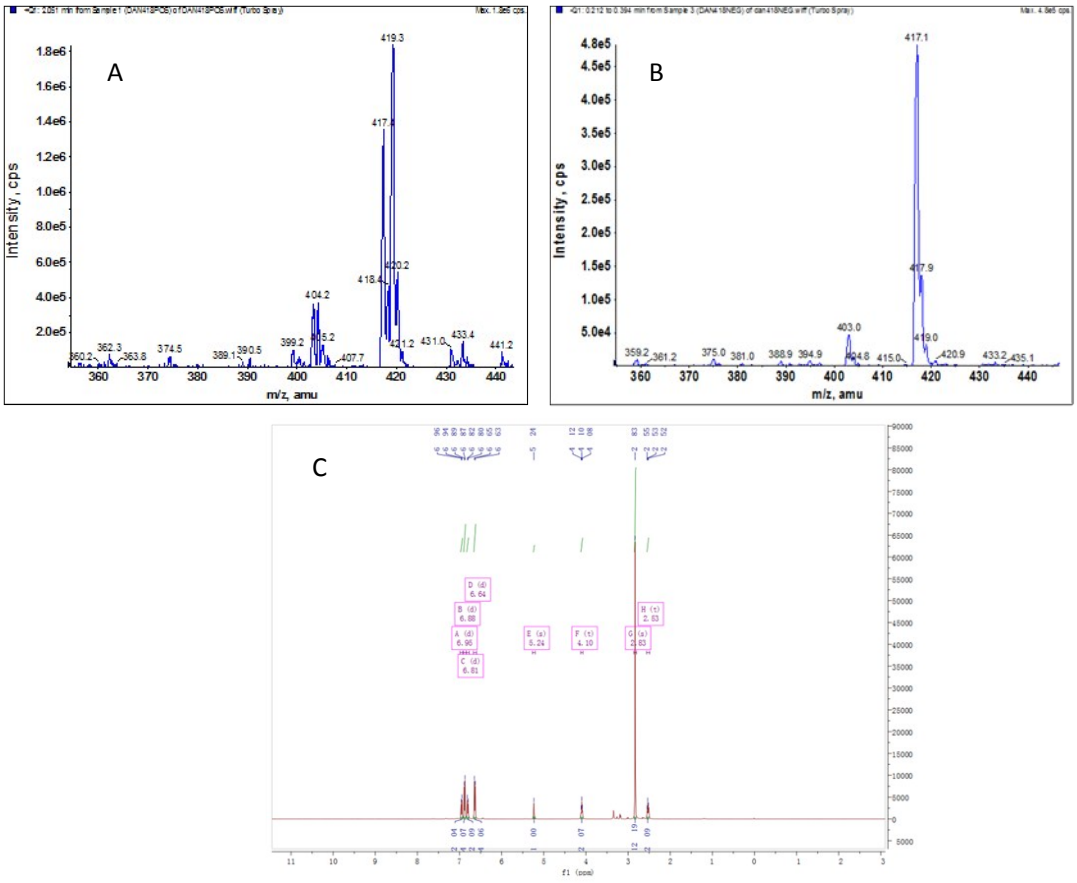

Figure S5. The results of leucomalachite green hapten IV (A- The mass spectrogram in positive mode; B- The mass spectrogram in negative mode; C-The NMR spectrum).

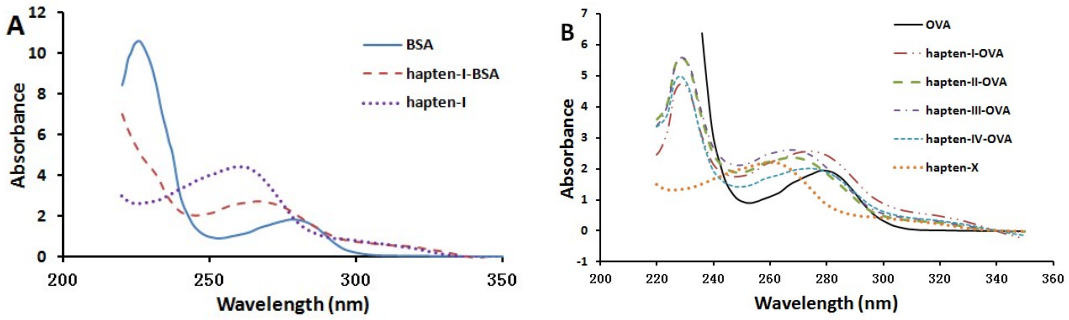

Figure S6. (A) UV spectra of LMG hapten I, BSA and immunogen hapten I-BSA. (B) UV spectra of LMG hapten I～IV (hapten-X), OVA and coating antigens hapten I-OVA, hapten II-OVA, hapten III-OVA, hapten IV-OVA.

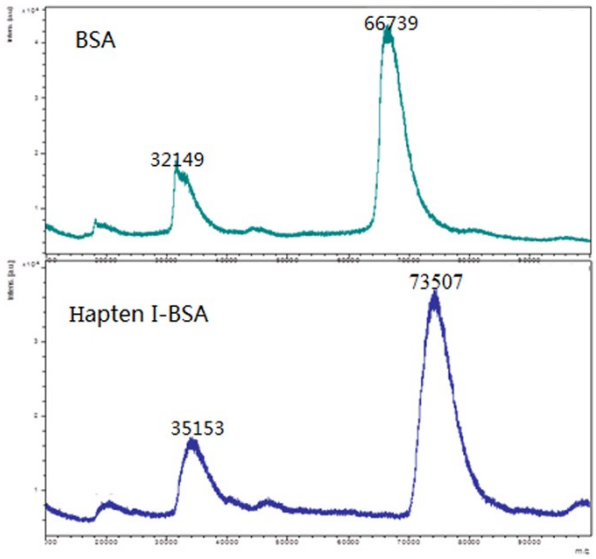

Figure S7. Mass spectra of the BSA and immunogen hapten I-BSA

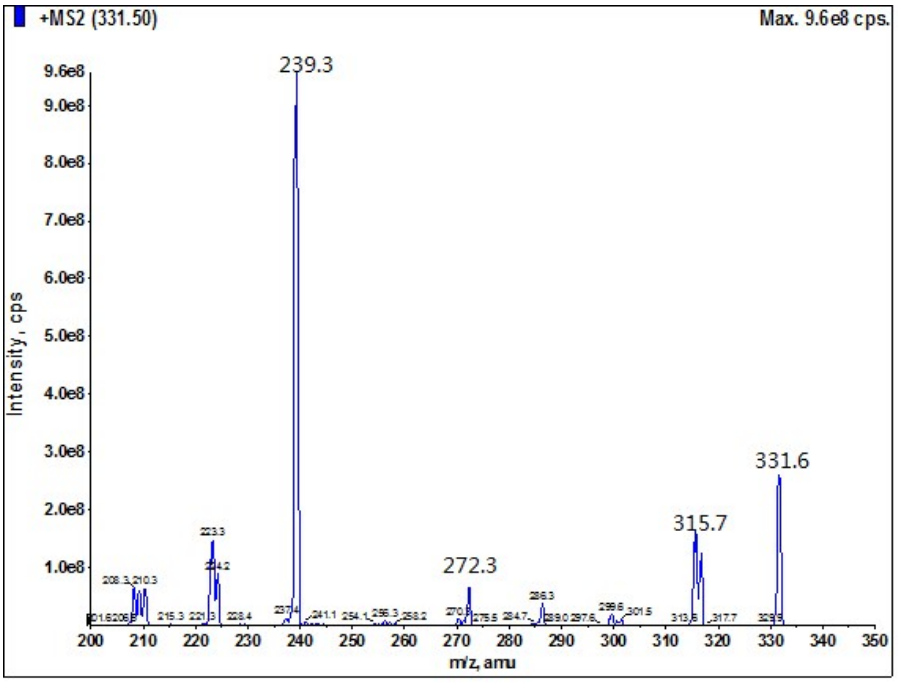

Figure S8. The product Ion spectrum of leucomalachite green at 500 μg/L.

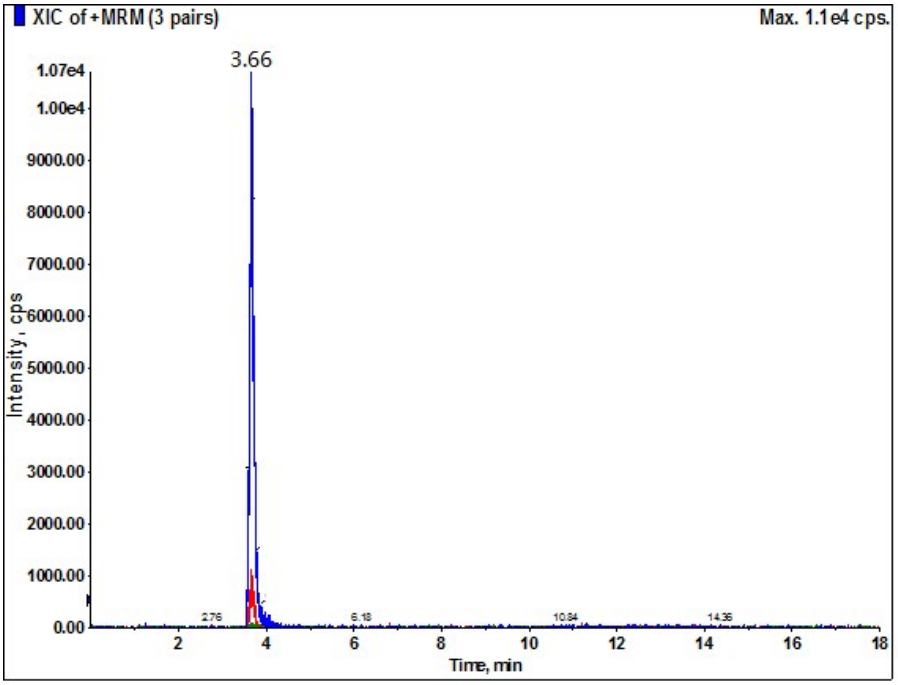

Figure S9. The MRM chromatogram of the leucomalachite green standard solution at 10 μg/L.