Electronic Supplementary Information (ESI)

Recovery of tetrodotoxin from pufferfish viscera extract by amine-functionalized magnetic nanocomposites

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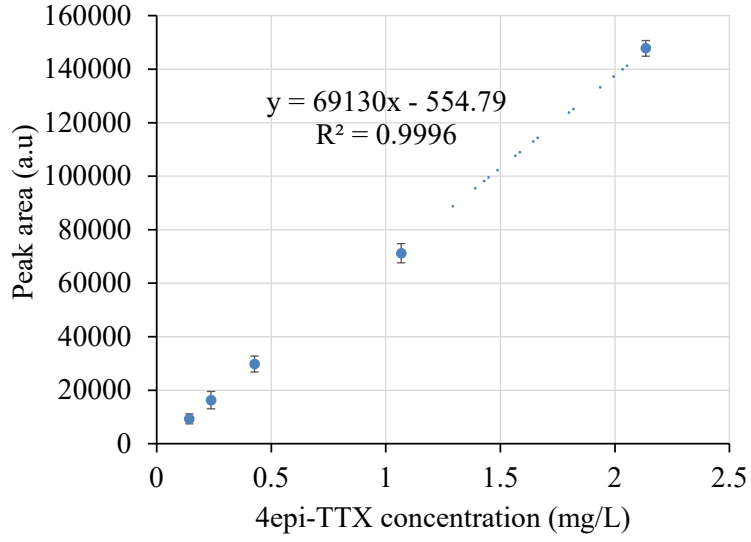
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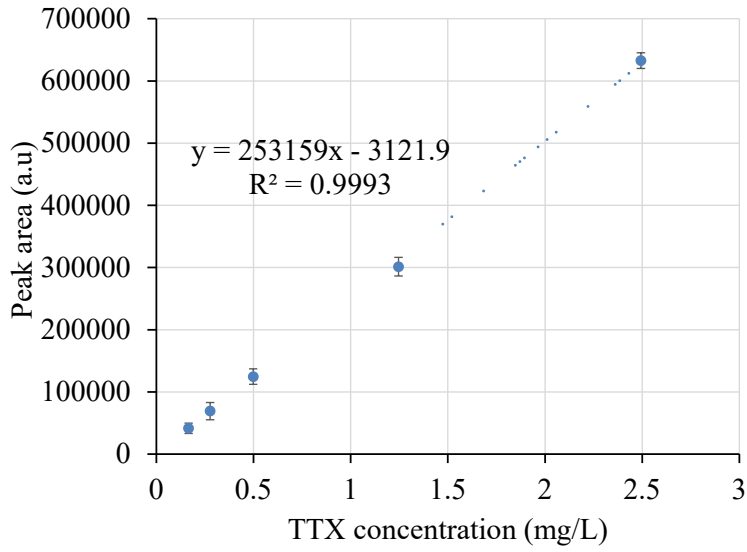
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Verification of HILIC-MS/MS methodology

The reliability of HILIC-MS/MS methodology for quantitative determination of 4epi-TTX, TTX and Anh-TTX in pufferfish viscera extract was evaluated in terms of linear range, correlation coefficients (R^2), the limits of detection (LODs) and quantification (LOQs). The real samples were purified according to the procedure that was described in **Section 2.6**. Calibrations curves were established by preparation of series of concentrations (0.1 to 10 mg/L) of the three analytes in acetonitrile/water/formic acid (700:300:0.1 v/v/v) solvent. The linear ranges for the three analytes were in the range of 0.1 - 5 mg/L with correlation efficient of 0.9992 - 0.9996 (**Fig. S1-S3 and Table S1**). Limit of detection (LOD) and quantification (LOQ) correspond to 3 and 10 times the signal-to-noise ratio for the three analytes were 0.010 - 0.012 mg/L and 0.041 - 0.054 mg/L (**Table S1**), respectively.

Moreover, the accuracy of the HILIC-MS/MS method was validated from spiking the three analytes at three fortification levels (e.g., 0.5, 1, 1.5 mg/L) (**Table S2**). The results showed that recoveries of the three analytes from spiked samples were determined between 95.7 and 103.4% with RSD ranging from 0.97 to 2.21% (**Table S2**), indicating that the HILIC-MS/MS methodology in quantification of 4epi-TTX, TTX and Anh-TTX in pufferfish viscera extract was highly precise.
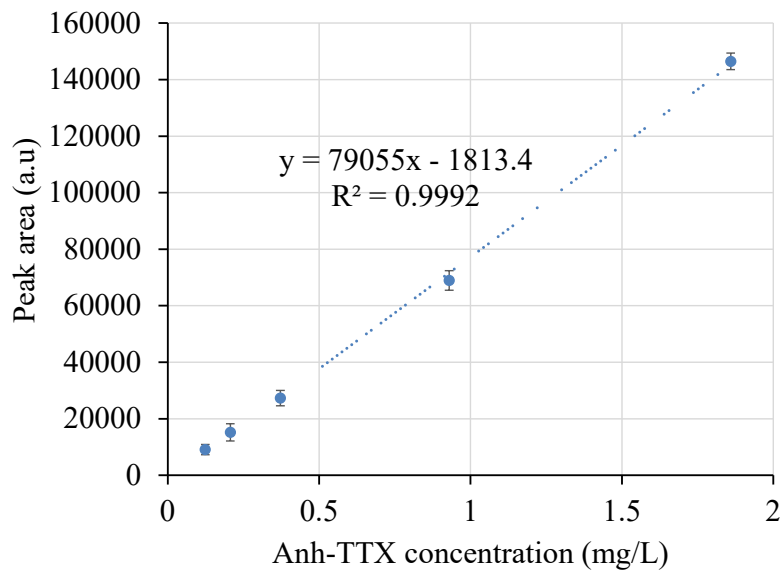
Standard curves

Fig. S1. Standard curve for 4epi-TTX.

In Fig. S1, the plot shows Peak area (a.u) on the y-axis versus 4epi-TTX concentration (mg/L) on the x-axis, with the equation:

$$y = 69130x - 554.79$$
$$R^2 = 0.9996$$

Fig. S2. Standard curve for TTX.

In Fig. S2, the plot shows Peak area (a.u) on the y-axis versus TTX concentration (mg/L) on the x-axis, with the equation:

$$y = 253159x - 3121.9$$
$$R^2 = 0.9993$$

Fig. S3. Standard curve for Anh-TTX.

Table S1. Linear range, regression coefficient, and the limits of detection and quantification of 4epi-TTX, TTX and Anh-TTX (n = 5).

Analytes	HILIC-MS/MS methodology			
	Linearity (mg/L)	R^2	LOD (mg/L)	LOQ (mg/L)
4epi-TTX	0.1 - 5	0.9996	0.010	0.054
TTX	0.1 - 5	0.9993	0.012	0.041
Anh-TTX	0.1 - 5	0.9992	0.011	0.047

Table S2. Accuracy of the HILIC-MS/MS methodology for the three analytes in real samples (pufferfish viscera extract).

Analytes	4epi-TTX		TTX		Anh-TTX	
Fortified content (mg/L)	Recovery (%) (n=5)	Inter-day precision (RSD, %) Intra-day (n=5)	Recovery (%) (n=5)	Inter-day precision (RSD, %) Intra-day (n=5)	Recovery (%) (n=5)	Inter-day precision (RSD, %) Intra-day (n=5)
0	ND	1.37	ND	0.98	ND	1.76
0.5	99.5	1.23	96.8	0.97	100.4	1.07
1.0	95.7	2.18	102.3	1.52	96.2	2.21
1.5	103.4	1.25	98.1	2.04	98.5	1.72

ND: Not determined