Supplementary Material

Determination of microcystins in water samples by deep eutectic solvent-based vortex-assisted liquid-liquid microextraction coupled with ultrahigh-performance liquid chromatography-high resolution mass spectrometry

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Table S1. Name (abbreviation), chemical structure and molecular weight of MC-LR and MC-YR

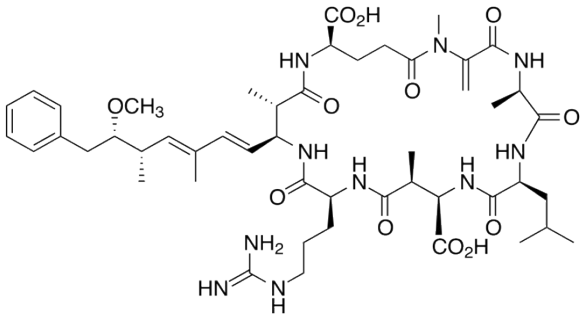
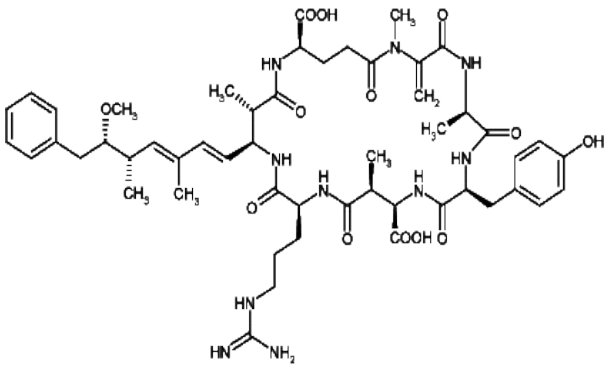
Name (Abbreviation):	**Structure**	**MW (g/mol)**	**pK_a**	**logK_{ow}**
Microcystin-LR (MC-LR)		**994.5488**	**3.3**	**4.2**
Microcystin-YR (MC-YR)		**1044.5280**	**3.9**	**3.4**

Table S2. Analysis of variance (ANOVA) of the Box-Behnken design for MC-LR peak areas

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Coefficient[a] (estimated)
Model	6.37E+09	9	7.07E+08	15.02	0.0009*	
Intercept						1.12E+05
A-THF volume	2.61E+07	1	2.61E+07	0.55	0.481	–1806
B-DES volume	3.12E+09	1	3.12E+09	67.12	< 0.0001*	19882
C-vortex time	4.27E+07	1	4.27E+07	0.91	0.3726	2311
AB	1.81E+08	1	1.81E+08	3.85	0.09	6736
AC	7.24E+07	1	7.24E+07	1.54	0.2552	–4253
BC	2.06E+06	1	2.06E+06	0.044	0.8402	–718
A^2	6.34E+08	1	6.34E+08	13.47	0.008	–12275
B^2	1.51E+09	1	1.51E+09	32.16	0.0008	–18969
C^2	4.53E+08	1	4.53E+08	9.61	0.0173	–10371
Residual	3.30E+08	7	4.71E+07			
Lack of Fit	1.35E+08	3	4.50E+07	0.92	0.5065	
R^2	0.9508					
Adjusted R^2	0.9078					
Predicted R^2	0.8852					

*Significant

[a] The estimated coefficients for the second order polynomial equation.

Table S3. Analysis of variance (ANOVA) of the Box-Behnken design for MC-YR peak areas

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Coefficient[a] (estimated)
Model	6.17E+09	9	6.86E+08	15.0	0.0009*	
Intercept						1.12E+05
A-THF volume	2.87E+07	1	2.87E+07	0.63	0.4544	–1893
B-DES volume	2.99E+09	1	2.99E+09	65.40	< 0.0001*	19336
C-vortex time	5.29E+07	1	5.29E+07	1.16	0.3180	2571
AB	1.32E+08	1	1.32E+08	2.88	0.1332	5743
AC	6.07E+07	1	6.07E+07	1.33	0.2870	–3897
BC	2.48E+06	1	2.48E+06	0.054	0.8225	–788
A^2	6.37E+08	1	6.37E+08	13.92	0.0073	–12296
B^2	1.54E+09	1	1.54E+09	33.66	0.0007	–19122
C^2	4.50E+08	1	4.50E+08	9.85	0.0164	–10343
Residual	3.20E+08	7	4.57E+07			
Lack of Fit	1.11E+08	3	3.70E+07	0.71	0.5951	
R^2	0.9507					
Adjusted R^2	0.9080					
Predicted R^2	0.8873					

*Significant

[a] The estimated coefficients for the second order polynomial equation.

Table S4. The variation of peak abundances: (+) peak enhancement/(−) peak suppression

Analytes	(+) peak enhancement/ (−) peak suppression (%)
MC-YR	+11*
MC-LR	+17

*The percentage of the "average peak abundances difference" (n = 3) obtained from the final extract divided by the peak abundance from the standard solution (5 ng/mL).

Table S5. Concentrations (ng/mL) of MC-YR and MC-LR detected in water samples from Shihmen Reservoir by using DES-VALLME coupled with UHPLC-qTOF-MS

Sample	**MC-YR**	**MC-LR**
Reservoir-1	n.d.	n.d.
Spiked recovery %	105[a] (3.6)[b]	109[a] (3.2)[b]
Reservoir-2	n.d.	n.d.
Spiked recovery %	106 (7.6)	98 (3.7)
Reservoir-3	n.d.	n.d.
Spiked recovery %	100 (12.2)	94.0 (10.4)
Reservoir-4	n.d.	n.d.
Spiked recovery %	98.7 (4.5)	95.6 (5.7)

n.d.: not detected.
[a] Average spiked recovery (trueness, %, n = 3; spiked final concentration: 2.0 ng/mL).
[b] Relative standard deviation (RSD) of spiked recovery (precision, %, n = 3).

Table S6. The penalty points for MC-YR and MC-LR determination by DES-VALLME plus UHPLC-ESI(+)-qTOF-MS

Reagents	penalty points
THF	1
Acetonitrile	1
Methanol	1
DES	1
Formic acid	2
Instruments	
LC-MS	2
Centrifuge	1
Vortex agitator	1
Waste	2
Total penalty points	12
Analytical Eco-scale total score = 88	

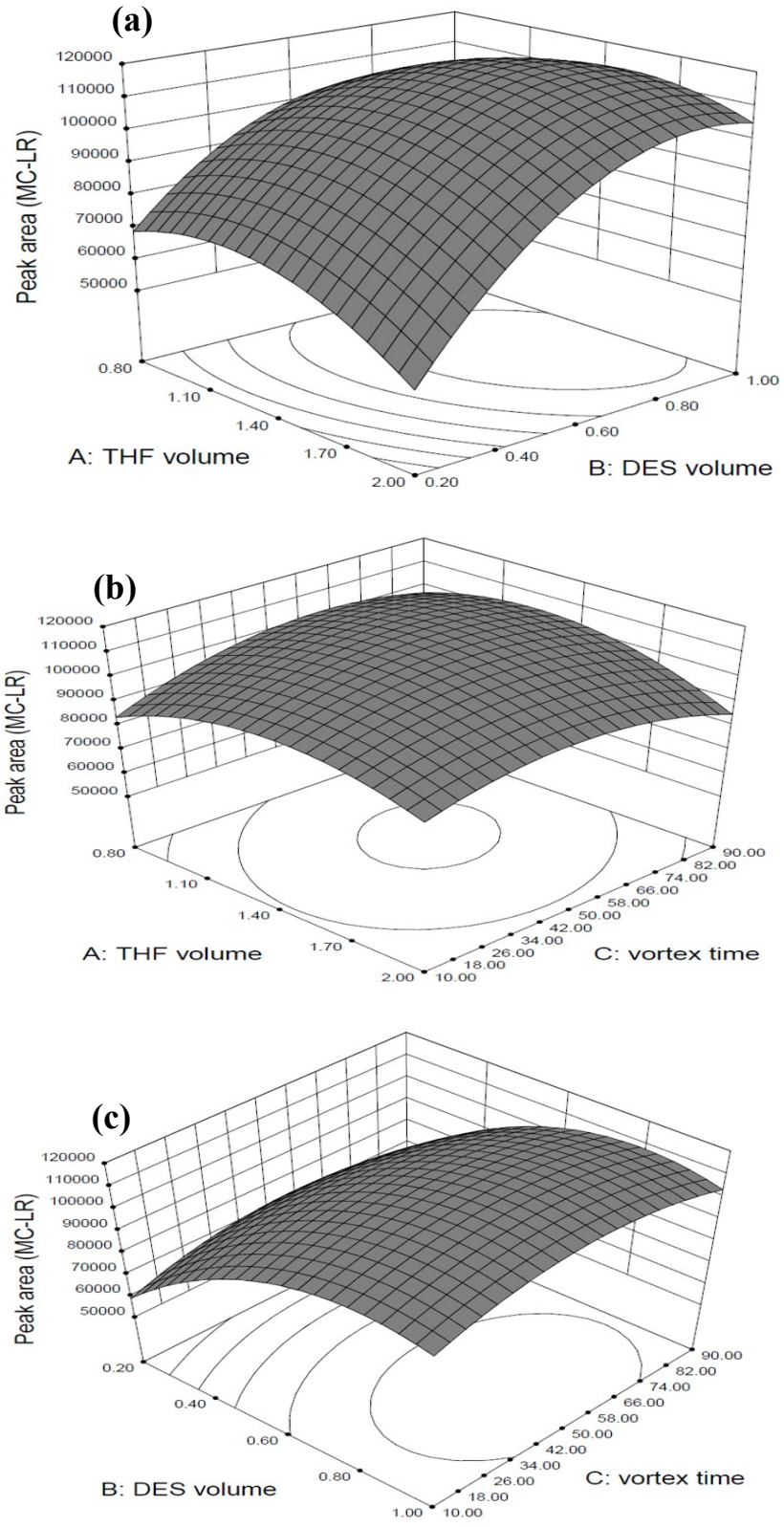


Figure S1. 3D response surface plots for peak area of MC-LR estimated from the BBD on each pair of independent variables: (a) volume of THF *vs*. volume of DES; (b) volume of THF *vs*. vortex-time; (c) volume of DES *vs*. vortex-time.

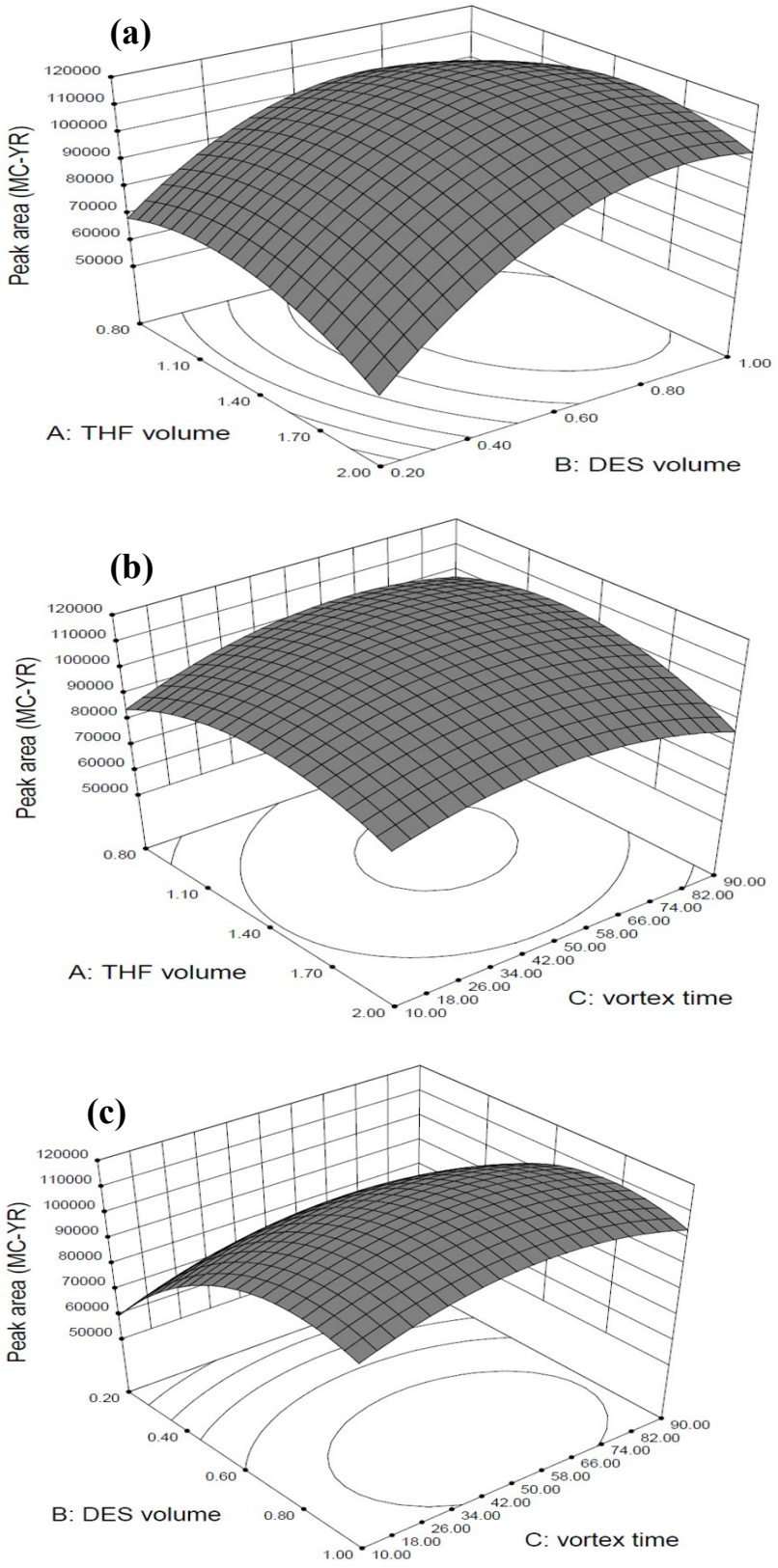


Figure S2. 3D response surface plots for peak area of MC-LR estimated from the BBD on each pair of independent variables: (a) volume of THF *vs*. volume of DES; (b) volume of THF *vs*. vortex-time; (c) volume of DES *vs*. vortex-time.

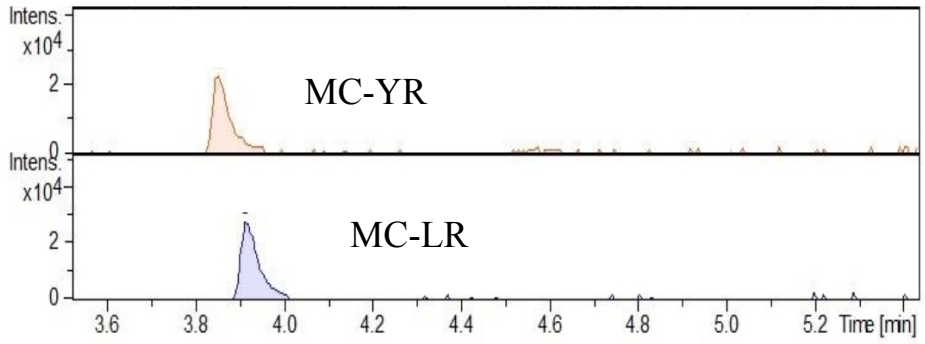


Figure S3. UHPLC-ESI(+)-qTOF-MS extracted ion chromatograms for a spiked water sample from "Reservoir" (spiked at the final concentrations of 2 ng/mL for each analyte).