

## Electronic Supplementary Information (ESI)

### **Analysis of canthin-6-one alkaloids derived from *Eurycoma* spp. by micellar liquid chromatography and conventional high-performance liquid chromatography: A comparative evaluation**

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**Table S1** Experimental design by Box-Behnken design (BBD) of response surface methodology (RSM)

Factors	Symbol	Unit	Level		
			Low (-1)	Medium (0)	High (+1)
<b>Independent variables</b>					
Sodium dodecyl sulfate concentration	$X_1$	mM	90	110	130
Acetonitrile concentration	$X_2$	vol%	5	10	15
pH	$X_3$	-	3	5	7
<b>Dependent variables</b>					
Retention time (C6OG)	$Y_1$	min			
Retention time (9HCO)	$Y_2$	min			
Retention time (C6O)	$Y_3$	min			
Retention time (9MCO)	$Y_4$	min			
Resolution of C6OG and 9HCO	$Y_5$	-			
Resolution of 9HCO and C6O	$Y_6$	-			
Resolution of C6O and 9MCO	$Y_7$	-			

**Table S2** Effect of sodium dodecyl sulfate (SDS) concentration on the elution of micellar liquid chromatography mobile phases

SDS concentration (mM) <sup>1</sup>	Retention time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG-HCO	HCO-C6O	C6O-MCO
30	8.13	37.56	61.22	74.03	21.31	5.85	3.08
60	5.27	19.54	31.04	37.29	16.65	7.93	2.53
90	4.27	14.31	23.02	26.01	14.47	7.27	2.34
120	3.67	11.4	18.13	21.03	12.91	6.76	2.14

<sup>1</sup>The mobile phase consisted of 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5)

**Table S3** Effect of cetyltrimethylammonium bromide (CTAB) concentration on the elution of micellar liquid chromatography mobile phases

CTAB concentration (mM) <sup>1</sup>	Retention time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG-HCO	HCO-C6O	C6O-MCO
30	9.54	51.95	51.95	57.62	17.41	-	1.39
60	6.74	27.94	27.94	30.67	14.95	-	1.2
90	5.4	19.73	19.73	21.62	12.6	-	1.07
120	4.65	15.51	15.51	16.97	11.25	-	0.98

<sup>1</sup>The mobile phase consisted of 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5)

**Table S4** Effect of Brij-35 concentration on the elution of micellar liquid chromatography mobile phases

Brij 35 concentration (mM) <sup>1</sup>	Retention time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG-HCO	HCO-C6O	C6O-MCO
12.5	9.72	71.52	>80	>80	29.04	-	-
25.0	7.87	40.08	49.94	57.15	22.56	4.14	2.56
37.5	6.69	28.19	35.59	40.02	18.94	4.04	2.04
50.0	5.83	21.82	27.70	30.85	16.45	3.80	1.72

<sup>1</sup>The mobile phase consisted of 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5)

**Table S5** Effect of pH on the elution of micellar liquid chromatography mobile phases with sodium dodecyl sulfate (SDS)

pH of mobile phase <sup>1</sup>	Retention Time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG- HCO	HCO-C6O	C6O-MCO
3.0	3.20	11.54	21.81	24.40	13.23	7.98	1.28
5.0	3.67	11.4	18.13	21.03	12.91	6.76	2.14
7.0	3.42	10.19	16.25	17.74	12.23	6.64	1.24

<sup>1</sup>The mobile phase consisted of 120 mM SDS and 10 mM NaH<sub>2</sub>PO<sub>4</sub>

**Table S6** Effect of pH on the elution of micellar liquid chromatography mobile phases with cetyltrimethylammonium bromide (CTAB)

pH of mobile phase <sup>1</sup>	Retention Time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG- HCO	HCO-C6O	C6O-MCO
3.0	4.43	15.34	15.91	17.46	7.82	-	0.78
5.0	4.65	15.51	15.51	16.97	11.25	-	0.98
7.0	C6OG	C6O	MCO	HCO	C6OG-C6O	C6O-MCO	MCO-HCO
	5.16	15.78	17.11	20.12	10.9	0.91	1.62

<sup>1</sup>The mobile phase consisted of 120 mM CTAB and 10 mM NaH<sub>2</sub>PO<sub>4</sub>

**Table S7** Effect of pH on the elution of micellar liquid chromatography mobile phases with Brij 35

pH of mobile phase <sup>1</sup>	Retention Time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG- HCO	HCO-C6O	C6O-MCO
3.0	5.72	25.77	33.89	37.55	16.63	3.79	1.39
5.0	6.69	28.19	35.59	40.02	18.94	4.04	2.04
7.0	6.54	26.39	34.47	38.59	15.70	3.80	1.60

<sup>1</sup>The mobile phase consisted of 37.5 mM Brij 35 and 10 mM NaH<sub>2</sub>PO<sub>4</sub>

**Table S8** Effect of additives on elution of micellar liquid chromatography mobile phases

Additives <sup>1</sup>	Retention Time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG- HCO	HCO-C6O	C6O-MCO
Acetonitrile	3.44	11.857	18.953	23.200	12.03	3.65	1.43
Butanol	2.723	7.977	12.107	13.447	10.16	3.47	-
Propanol	3.073	9.963	16.420	19.100	10.39	3.12	0.77
Propylene glycol	3.323	11.717	20.320	24.493	9.86	3.00	-

<sup>1</sup>The mobile phase consisted of 120 mM sodium dodecyl sulfate (SDS) and 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5). The mobile phase investigated all additives at 4% (v/v).

**Table S9** Effect of acetonitrile concentration on elution of micellar liquid chromatography mobile phases

Acetonitrile concentration	Retention Time (min)				Resolution		
	C6OG	HCO	C6O	MCO	C6OG- HCO	HCO-C6O	C6O-MCO
2%	3.637	13.130	21.383	25.880	10.56	2.86	0.94
4%	3.44	11.857	18.953	23.200	12.03	3.65	1.43
10%	3.150	9.983	16.707	19.393	10.97	4.67	1.25
15%	2.683	8.133	14.477	16.340	9.00	4.86	0.95
20%	2.337	6.010	11.337	12.510	12.59	6.12	0.89

<sup>†</sup>The mobile phase consisted of 120 mM sodium dodecyl sulfate (SDS) and 10 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5).

**Table S10** Effect of mobile phase composition from Box-Benhen Design of response surface methodology on the elusion of micellar liquid chromatography

Experiments	Independent factors			Dependent factors						
	$X_1$	$X_2$	$X_3$	$Y_1$	$Y_2$	$Y_3$	$Y_4$	$Y_5$	$Y_6$	$Y_7$
1	90	5	5	4.34	16.65	27.32	33.62	10.02	3.54	1.37
2	130	5	5	3.50	11.85	19.00	22.70	9.88	3.65	1.25
3	90	15	5	3.06	10.46	19.22	22.01	9.04	4.52	0.96
4	130	15	5	2.80	8.37	14.66	16.51	9.25	4.80	0.94
5	90	10	3	5.48	23.02	45.50	53.15	12.66	6.83	1.60
6	130	10	3	4.20	16.71	32.32	37.90	11.46	6.31	1.57
7	90	10	7	3.52	11.05	20.54	21.86	6.63	2.63	0.42
8	130	10	7	3.09	8.76	15.68	16.44	9.30	3.14	0.35
9	110	5	3	4.06	17.58	32.83	40.42	13.74	5.98	1.98
10	110	15	3	3.63	16.16	25.43	35.90	12.48	4.13	3.16
11	110	5	7	3.67	11.40	20.58	21.58	9.06	2.67	0.63
12	110	15	7	2.82	8.06	15.27	16.37	9.31	3.53	0.70
13	110	10	5	3.34	11.15	18.43	22.48	8.57	3.45	1.33
14	110	10	5	3.30	10.97	18.31	22.29	8.84	3.59	1.34
15	110	10	5	3.39	11.18	18.21	22.11	8.99	3.31	1.25
16	110	10	5	3.39	11.18	18.25	22.12	8.70	3.32	1.24
17	110	10	5	3.39	10.96	18.19	21.99	8.85	3.85	1.42



**Table S11** ANOVA data of regression parameters for retention time of analytes

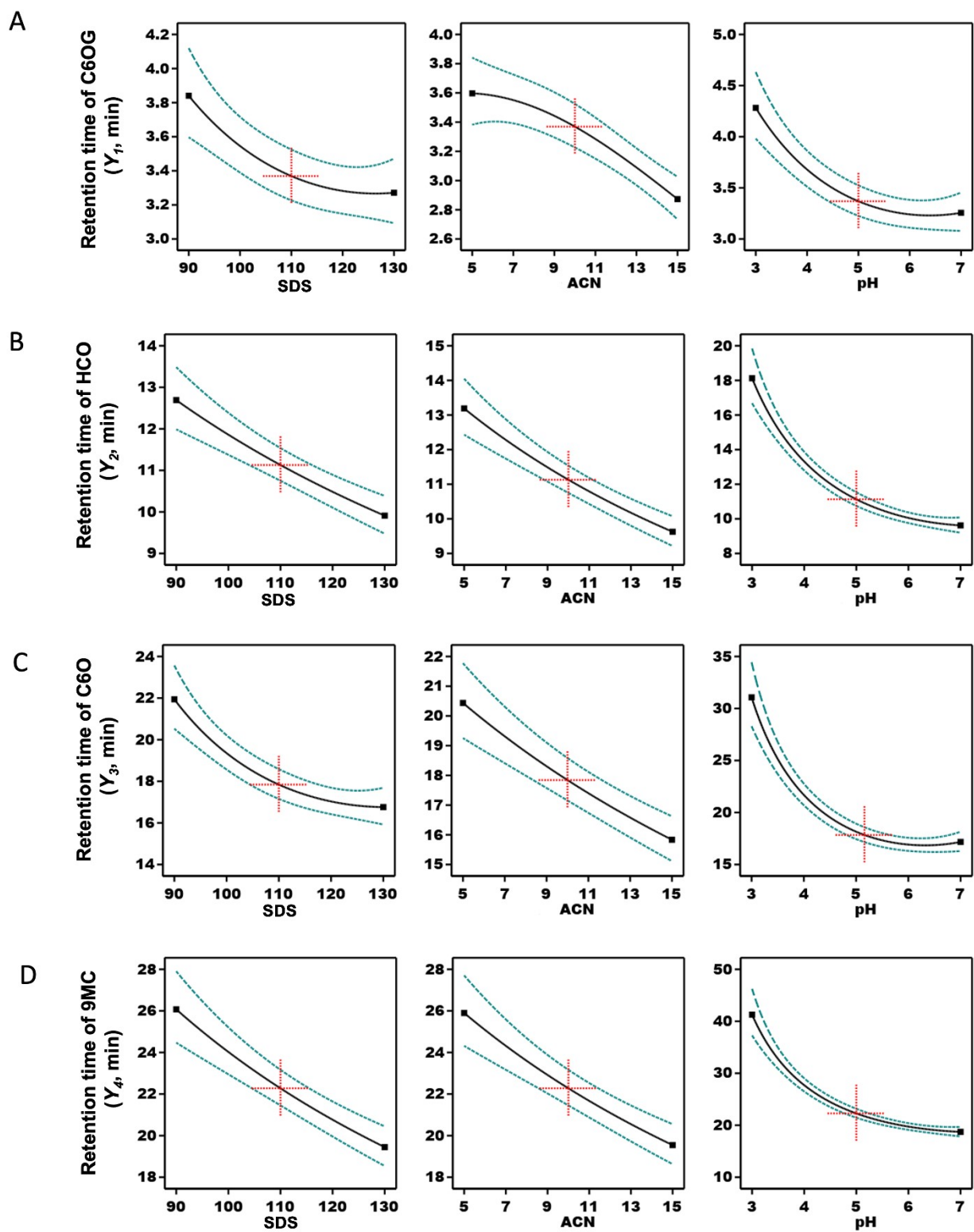
Source	$Y_1$			$Y_2$			$Y_3$			$Y_4$		
	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	Reduced quadratic model (Transform: Inverse)			Reduced quadratic model (Transform: Inverse)			Reduced quadratic model (Transform: Inverse)			Reduced quadratic model (Transform: Inverse)		
	0.0301	25.61	<0.0001	0.0080	85.47	<0.0001	0.0026	79.81	<0.0001	0.0025	92.25	<0.0001
$X_1$	0.0041	24.24	0.0008	0.0010	52.30	<0.0001	0.0004	60.22	<0.0001	0.0003	61.93	<0.0001
$X_2$	0.0098	58.36	<0.0001	0.0016	84.17	<0.0001	0.0004	61.38	<0.0001	0.0003	57.14	<0.0001
$X_3$	0.0108	64.14	<0.0001	0.0047	253.74	<0.0001	0.0013	205.23	<0.0001	0.0017	307.90	<0.0001
$X_1X_2$	-	-	-	-	-	-	-	-	-	-	-	-
$X_1X_3$	-	-	-	-	-	-	-	-	-	-	-	-
$X_2X_3$	0.0007	4.27	0.0686	0.0002	13.24	0.0039	-	-	-	0.0000	6.18	0.0303
$X_1^2$	0.0008	4.71	0.0581	-	-	-	0.0000	7.51	0.0192	-	-	-
$X_2^2$	0.0011	6.62	0.0300	-	-	-	-	-	-	-	-	-
$X_3^2$	0.0029	17.55	0.0023	0.0004	23.88	0.0005	0.0004	62.08	<0.0001	0.0002	28.11	0.0003
Residual	0.0015			0.0002			0.0001			0.0001		
Lack of Fit	0.0015	23.74	0.0044	0.0002	33.80	0.0021	0.0001	124.57	0.0002	0.0001	56.74	0.0008
Pure Error	0.0000			3.404E-06			3.297E-07			6.015E-07		
Cor Total	0.0316			0.0082			0.0027			0.0026		

**Table S12** ANOVA data of regression parameters for resolution of analytes

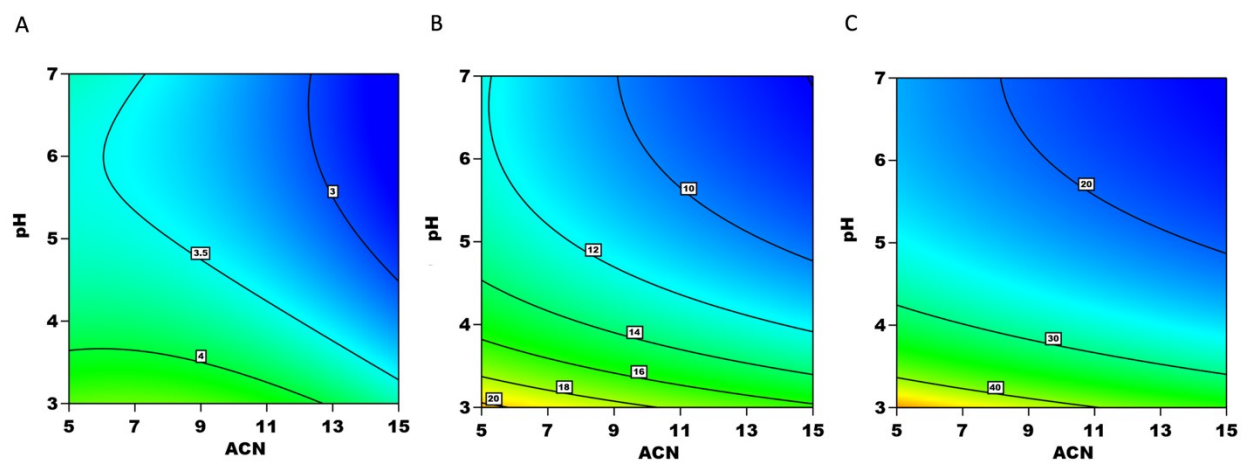
Source	$Y_5$			$Y_6$			$Y_7$		
	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	Reduced quadratic model (Transform: Inverse Sqrt)			Reduced 2FI model (Transform: Inverse Sqrt)			Reduced quadratic model (Transform: Inverse Sqrt)		
	0.0120	33.64	<0.0001	0.0603	19.44	<0.0001	1.28	30.19	<0.0001
$X_1$	0.0003	4.77	0.0539	-	-	-	0.0053	0.7490	0.4069
$X_2$	0.0002	2.74	0.1288	0.0019	1.88	0.1935	0.0010	0.1450	0.7113
$X_3$	0.0076	128.00	<0.0001	0.0518	50.04	<0.0001	0.9987	141.47	<0.0001
$X_1X_2$	-	-	-	-	-	-	-	-	-
$X_1X_3$	0.0014	23.49	0.0007	-	-	-	-	-	-
$X_2X_3$	-	-	-	0.0066	6.41	0.0250	-	-	-
$X_1^2$	-	-	-	-	-	-	0.1298	18.33	0.0016
$X_2^2$	0.0012	19.42	0.0013	-	-	-	0.0406	5.75	0.0374
$X_3^2$	0.0013	21.05	0.0010	-	-	-	0.1039	14.73	0.0033
Residual	0.0006			0.0134		-	0.0705		
Lack of Fit	0.0006	9.78	0.0224	0.0123	4.93	0.0695	0.0682	19.80	0.0061
Pure Error	0.0000			0.0011			0.0023		
Cor Total	0.0126			0.0738			1.35		

**Table S13** Fit statistics and equations for retention time and resolution of analytes

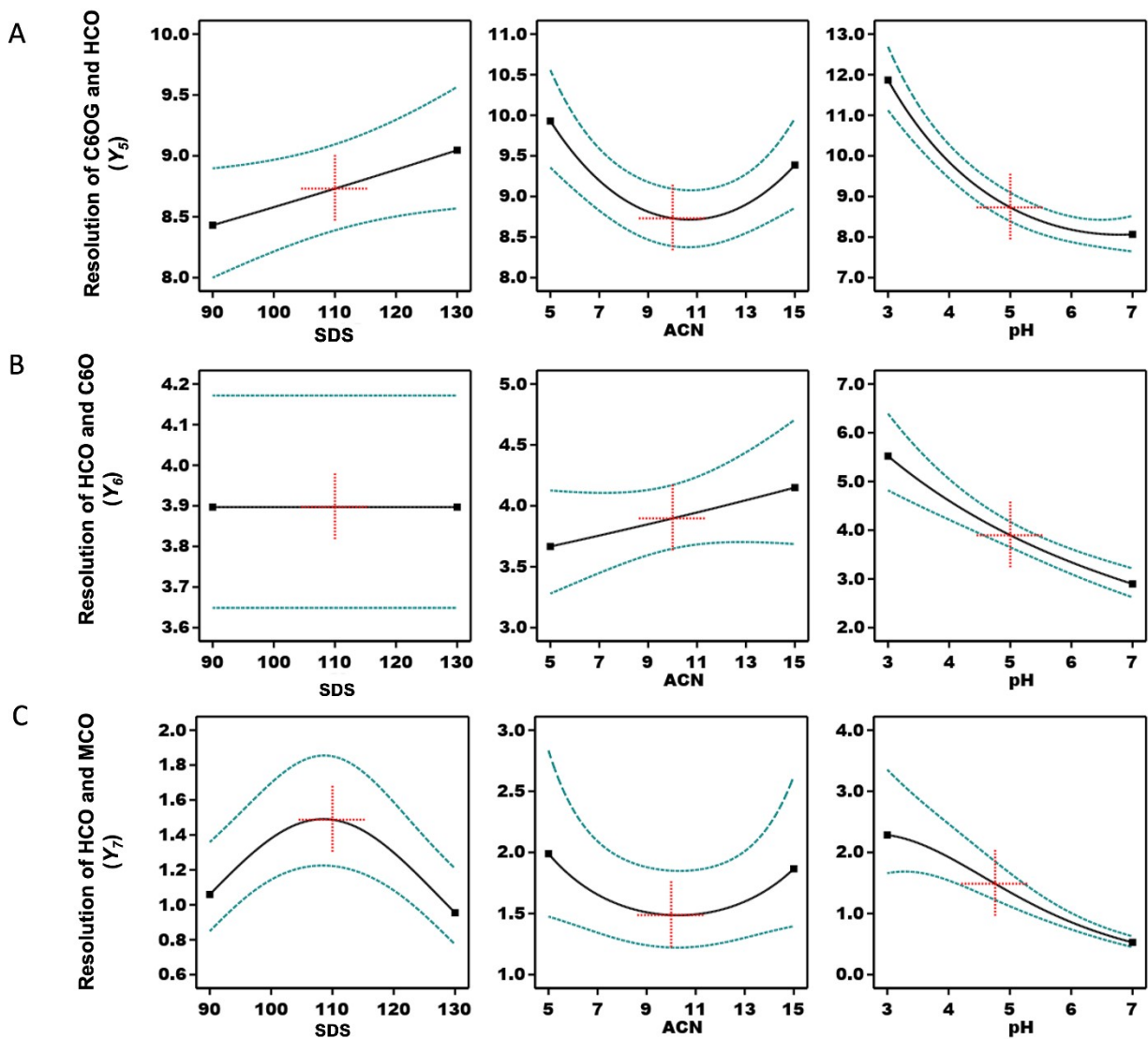
Response	Std. Dev.	Mean	C.V. %	$R^2$	Adjusted	Predicted	Coded equation
					$R^2$	$R^2$	
$Y_1$	0.0130	0.2861	4.53	0.9522	0.9150	0.5782	$1/Y_1 = 0.2974 + 0.0226X_1 + 0.0350X_2 + 0.0367X_3 + 0.0134(X_2 X_3) - 0.0137X_1^2 + 0.0163(X_2^2) - 0.0265(X_3^2)$
$Y_2$	0.0043	0.0852	5.06	0.9749	0.9635	0.8870	$1/Y_2 = 0.0901 + 0.0110X_1 + 0.0140X_2 + 0.0243X_3 + 0.0078X_2X_3 - 0.0102X_3^2$
$Y_3$	0.0026	0.0490	5.23	0.9732	0.9610	0.9171	$1/Y_3 = 0.0552 + 0.0070X_1 + 0.0071X_2 + 0.0130X_3 - 0.0034X_1^2 - 0.0098X_3^2$
$Y_4$	0.0023	0.0422	5.55	0.9767	0.9661	0.9066	$1/Y_4 = 0.0450 + 0.0065X_1 + 0.0063X_2 + 0.0145X_3 + 0.0029X_2X_3 - 0.0060X_3^2$
$Y_5$	0.0077	0.3228	2.39	0.9528	0.9245	0.7481	$1/\text{Sqrt}(Y_5) = 0.3387 - 0.0060X_1 + 0.0045X_2 + 0.0309X_3 - 0.0187X_1X_3 - 0.0165X_2^2 - 0.0172X_3^2$
$Y_6$	0.0322	0.5096	6.31	0.8178	0.7757	0.6183	$1/\text{Sqrt}(Y_6) = 0.5096 - 0.0156X_2 + 0.0804X_3 - 0.0407X_2X_3$
$Y_7$	0.0840	0.9833	8.54	0.9477	0.9163	0.7949	$1/\text{Sqrt}(Y_6) = 0.8732 + 0.0257X_1 + 0.0113X_2 + 0.3533X_3 + 0.1752X_1^2 - 0.0982X_2^2 + 0.1571X_3^2$



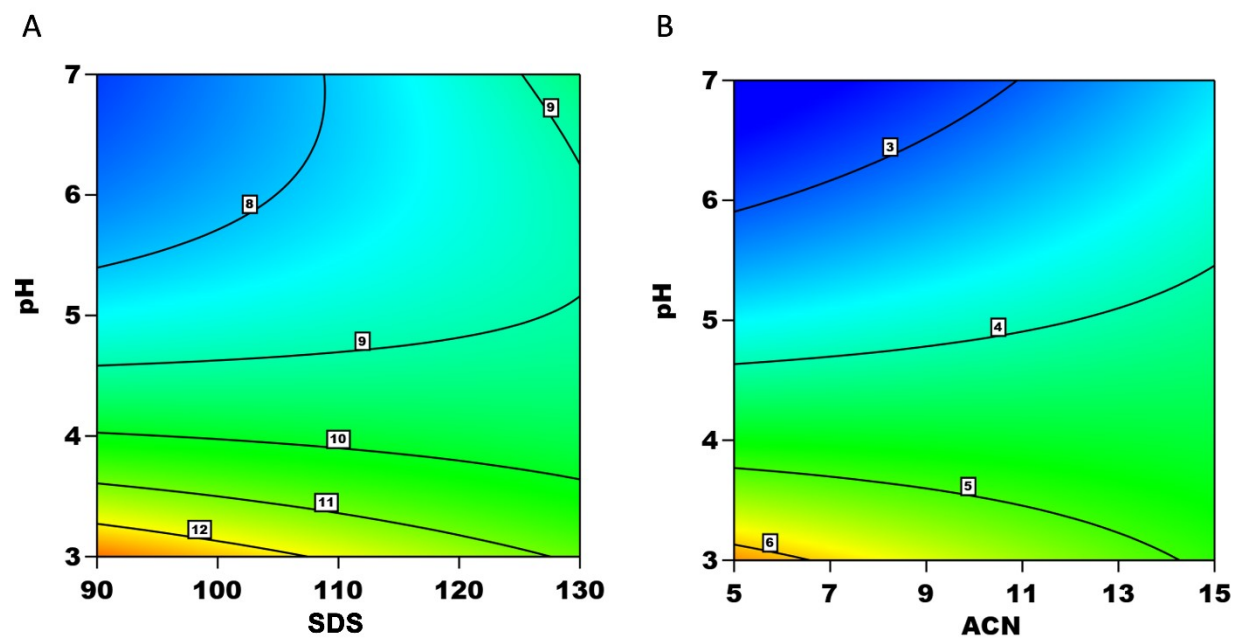
**Fig. S1** Effects of one factor on the response ( $Y_1$ – $Y_4$ , A–D, respectively), which the factors were included SDS concentration (mM), acetonitrile concentration (vol%), and pH.



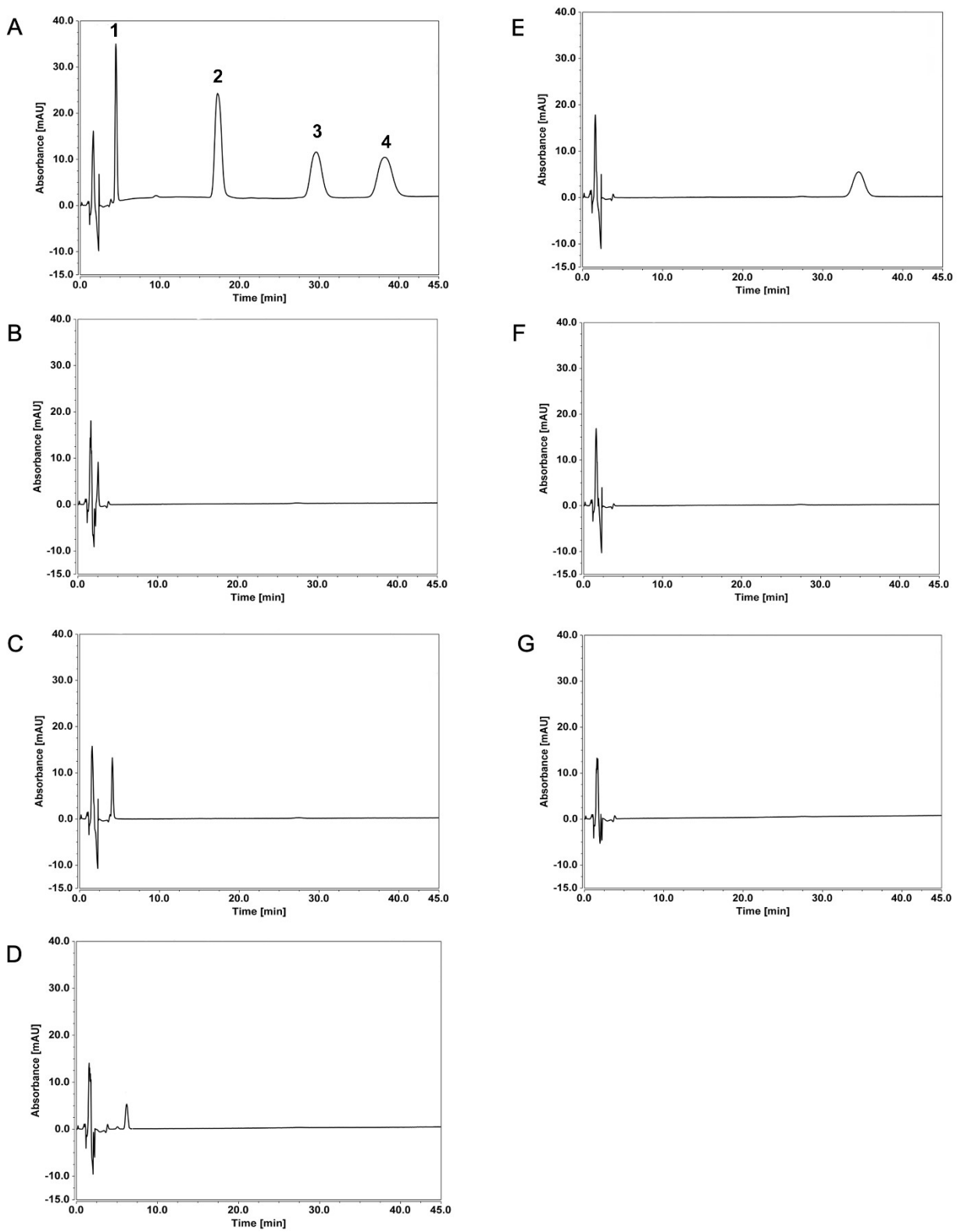
**Fig. S2** Contour plot represent the response plotted against combinations of acetonitrile concentration and pH of mobile phase, which A, B, and C are reponses  $Y_1$ ,  $Y_2$ , and  $Y_4$ , respectively.



**Fig. S3** Effects of one factor on the response ( $Y_5 - Y_7$ , A - C, respectively), which the factors were included SDS concentration (mM), acetonitrile concentration (vol%), and pH.



**Fig. S4** Contour plot represent the response plotted against combinations of SDS concentration and pH (A) and acetonitrile concentration and pH (B), which A and B are reponses  $Y_5$  and  $Y_6$ , respectively.



**Fig. S5** Chromatograms of analytes (A: 1, C6OG: 2, HCO: 3, C6O: 4, MCO), and other compounds of EL roots (B, CHA: C, SCO: D, EUL: E, CPA: F, 13-EEU: G, EU). All compounds were analyzed at 50  $\mu\text{g}/\text{mL}$  and detected at 272 nm.