Electronic Supplementary Information

Ultrasound-assisted combined with manganese-oxide nanoparticles loaded on activated carbon for extraction and pre-concentration of thymol and carvacrol in methanolic extracts of *Thymus daenensis, Salvia officinalis, Stachys pilifera, Satureja khuzistanica,* mentha, and water samples

Arash Asfaram [*a], Hossein Sadeghi [a], Alireza Goudarzi [b], Esmaeel Panahi kokhdan [a] and Zeinab Salehpour [a]

[a] *Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.*

[b] *Department of Polymer Engineering, Golestan University, Gorgan 49188-88369, Iran.*

[*] Corresponding author: E-mail address: arash.asfaram@yums.ac.ir (A. Asfaram)

Contents

1. Figures

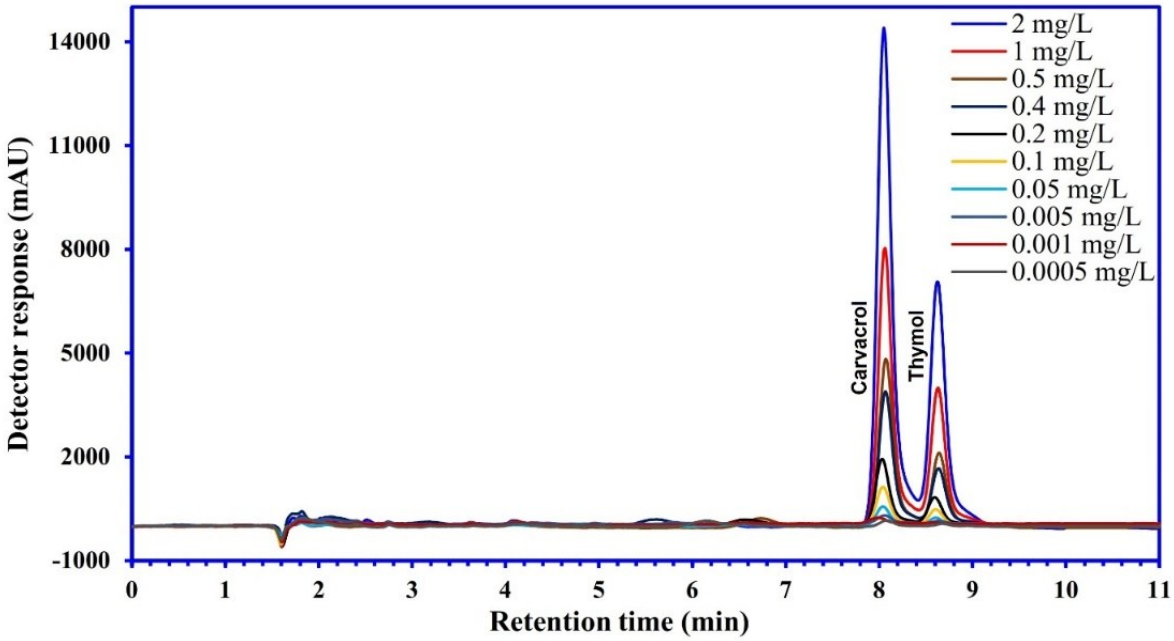

Figure S1. Calibration curve of carvacrol and thymol (mobile phase: acetonitrile-water (55:45, v/v); Flow rate: 1.1 mL min^{-1}; KNAUER Smart line HPLC system with 2500 basic model UV detector, column (4.6 mm diameter × 250 mm length, particle size of 5 µm, with a pre-column (Eurospher 100-5 C18)) under ambient temperature (25 °C); λ = 220 nm).

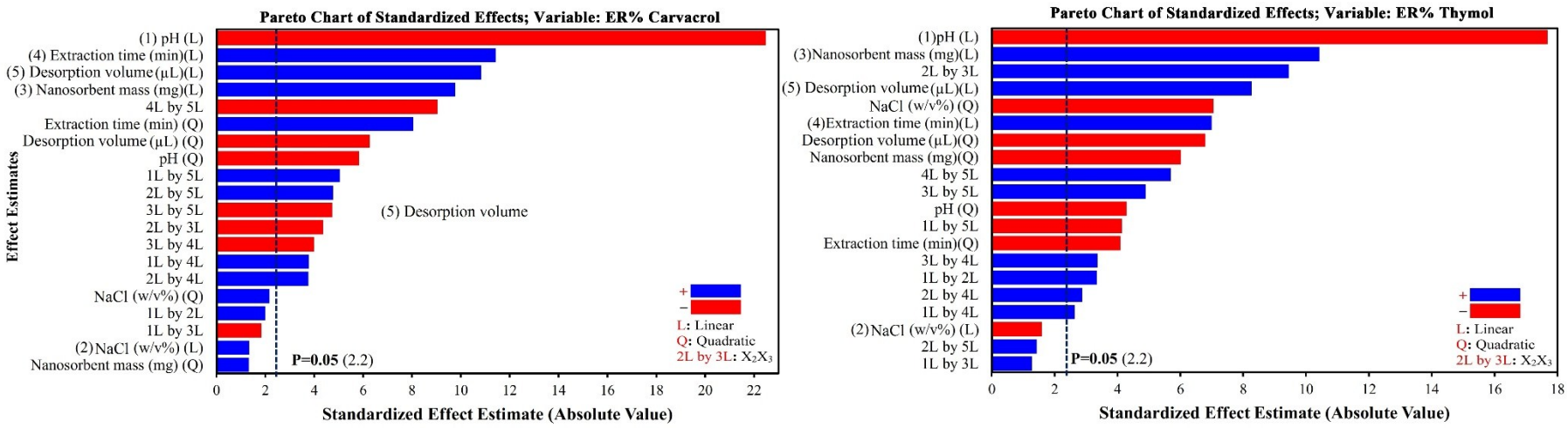

Figure S2. Pareto charts for the effect estimation. The effects presenting probability values higher than 0.05 were not considered as statistically significant.

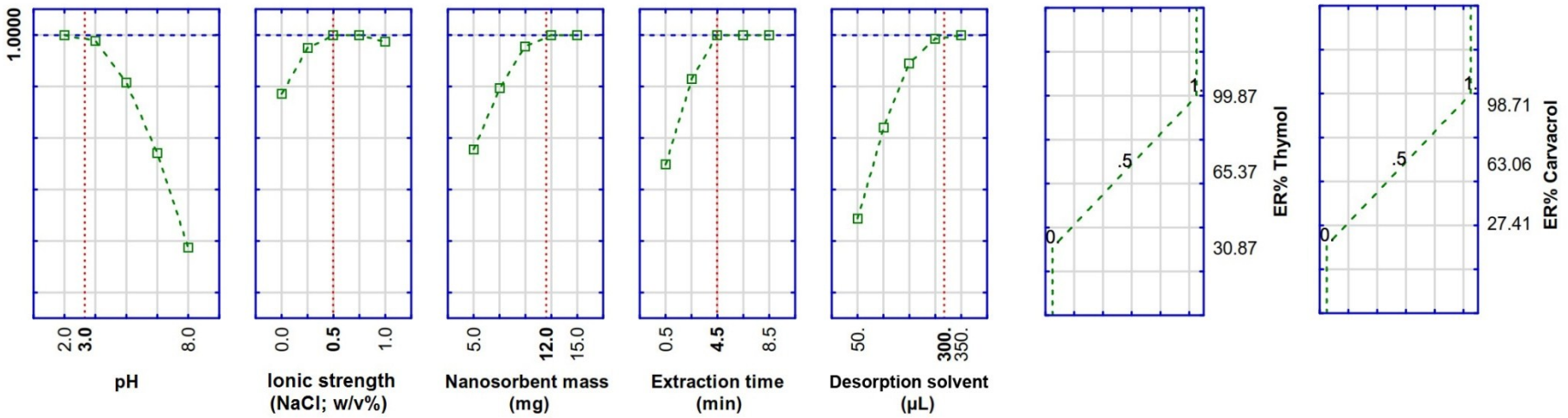

Figure S3. The conditions of profile optimum by the CCD design for the recovery of thymol and carvacrol.

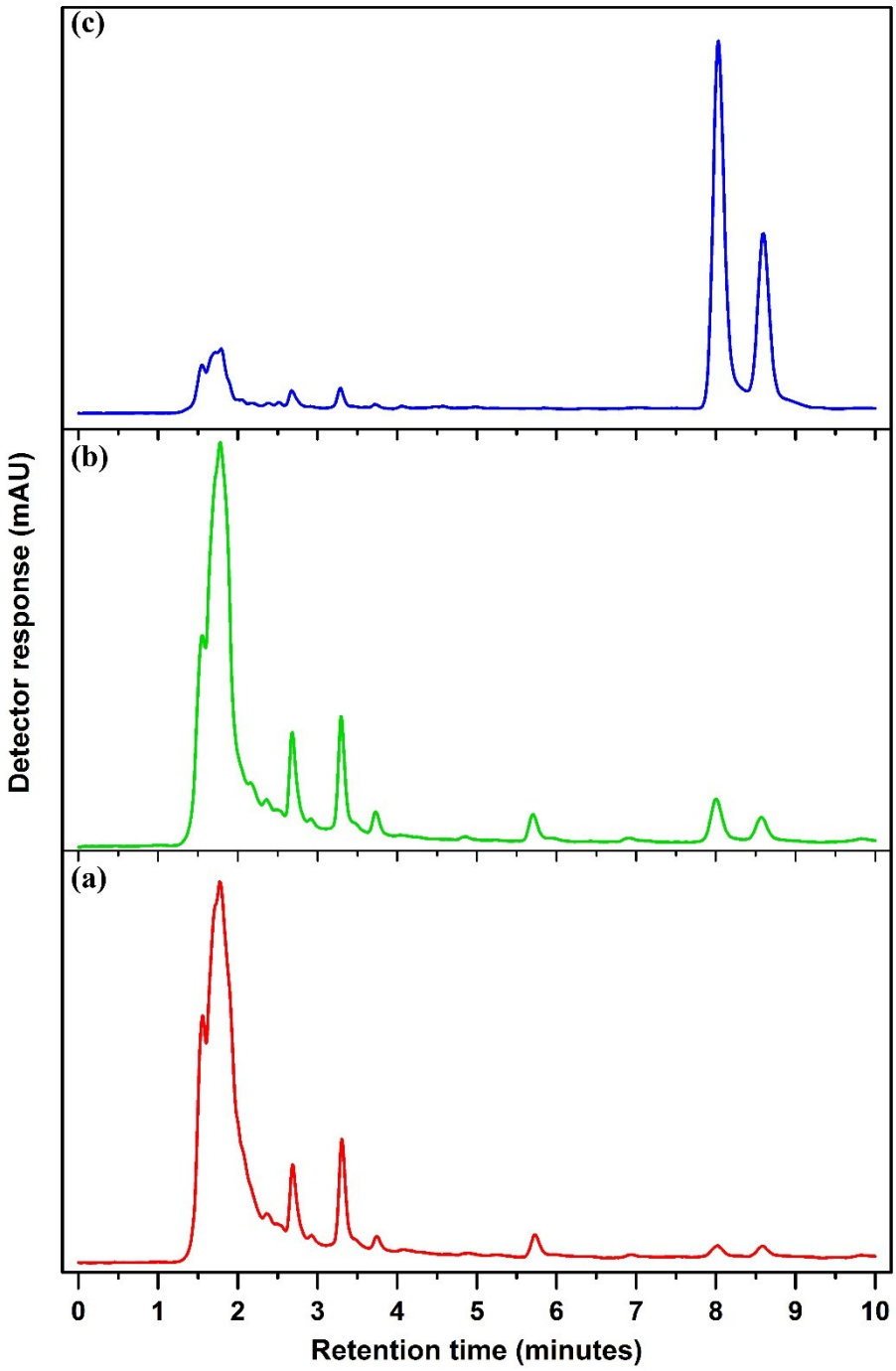

Figure S4. Typical chromatogram obtained for the extraction of *Thymus daenensis Celak* sample spiked with the two analytes under optimum conditions: (a) non-spiked, (b) spiked with 100 ng mL^{-1} before DMSPE and (c) extracted from water sample after DMSPE of analytes.

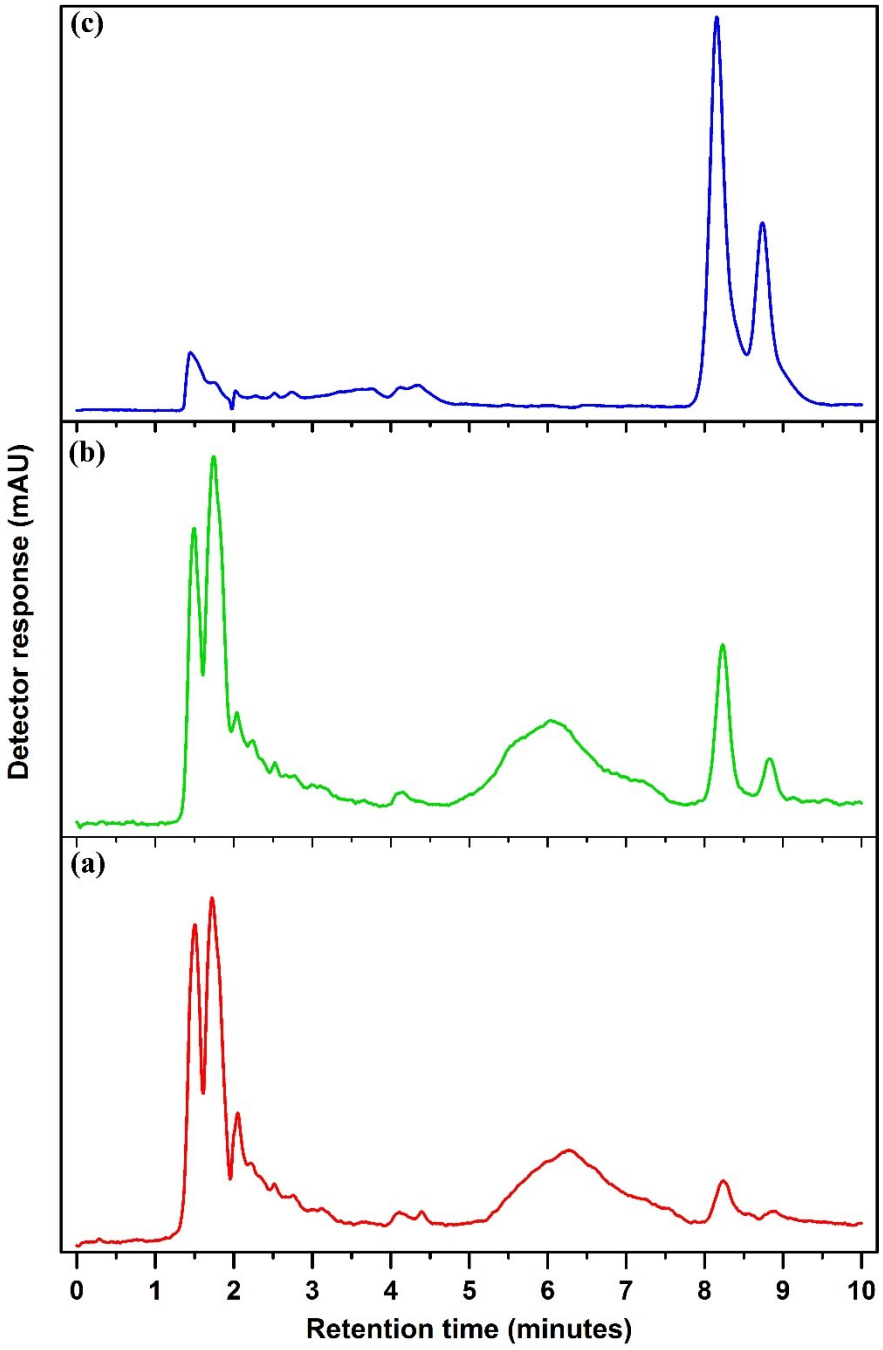

Figure S5. The chromatogram obtained by DMSPE-HPLC-UV for *Salvia officinalis* under optimum conditions: (a) non-spiked, (b) spiked with 100 ng mL^{-1} before DMSPE and (c) extracted from *Salvia officinalis* sample after DMSPE of thymol and carvacrol.

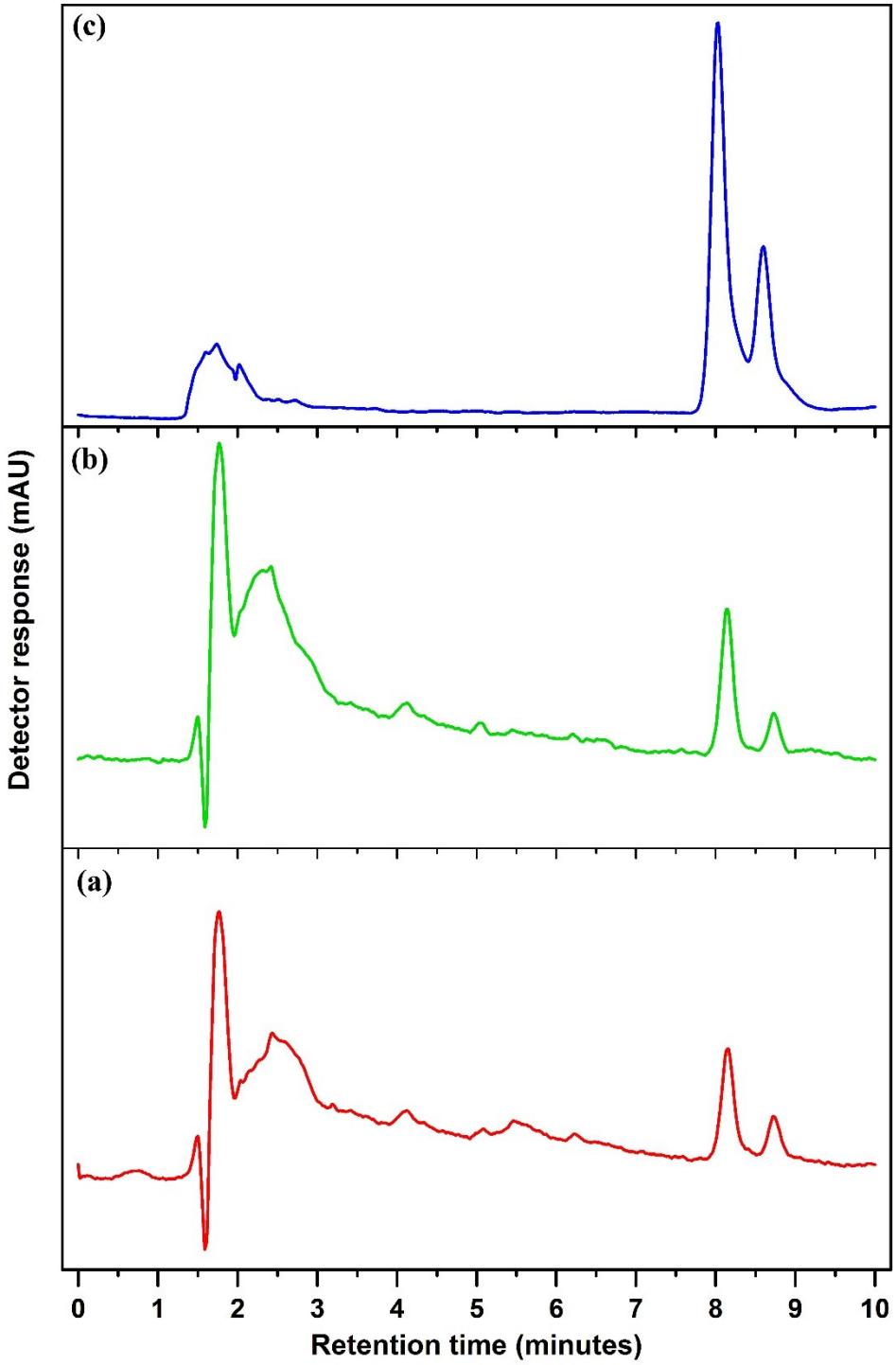

Figure S6. The chromatogram obtained by DMSPE-HPLC-UV for *Satureja khuzestanica* under optimum conditions: (a) non-spiked, (b) spiked with 100 ng mL^{-1} before DMSPE and (c) extracted from *Satureja khuzestanica* sample after DMSPE of analytes.

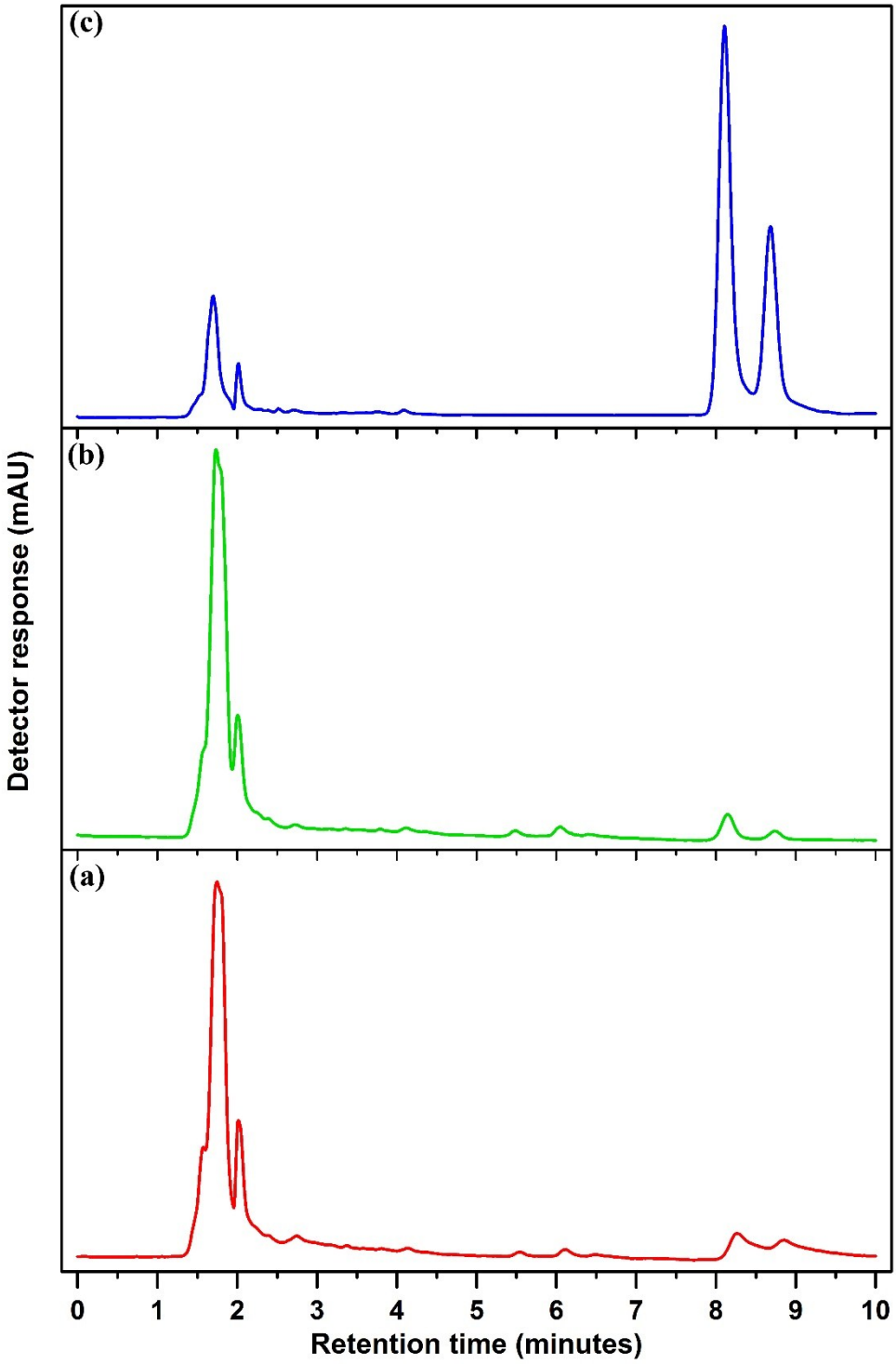

Figure S7. The chromatogram obtained by DMSPE-HPLC-UV for *Stachys pilifera* under optimum conditions: (a) non-spiked, (b) spiked with 100 ng mL^{-1} before DMSPE and (c) extracted from *Stachys pilifera* sample after DMSPE of thymol and carvacrol.

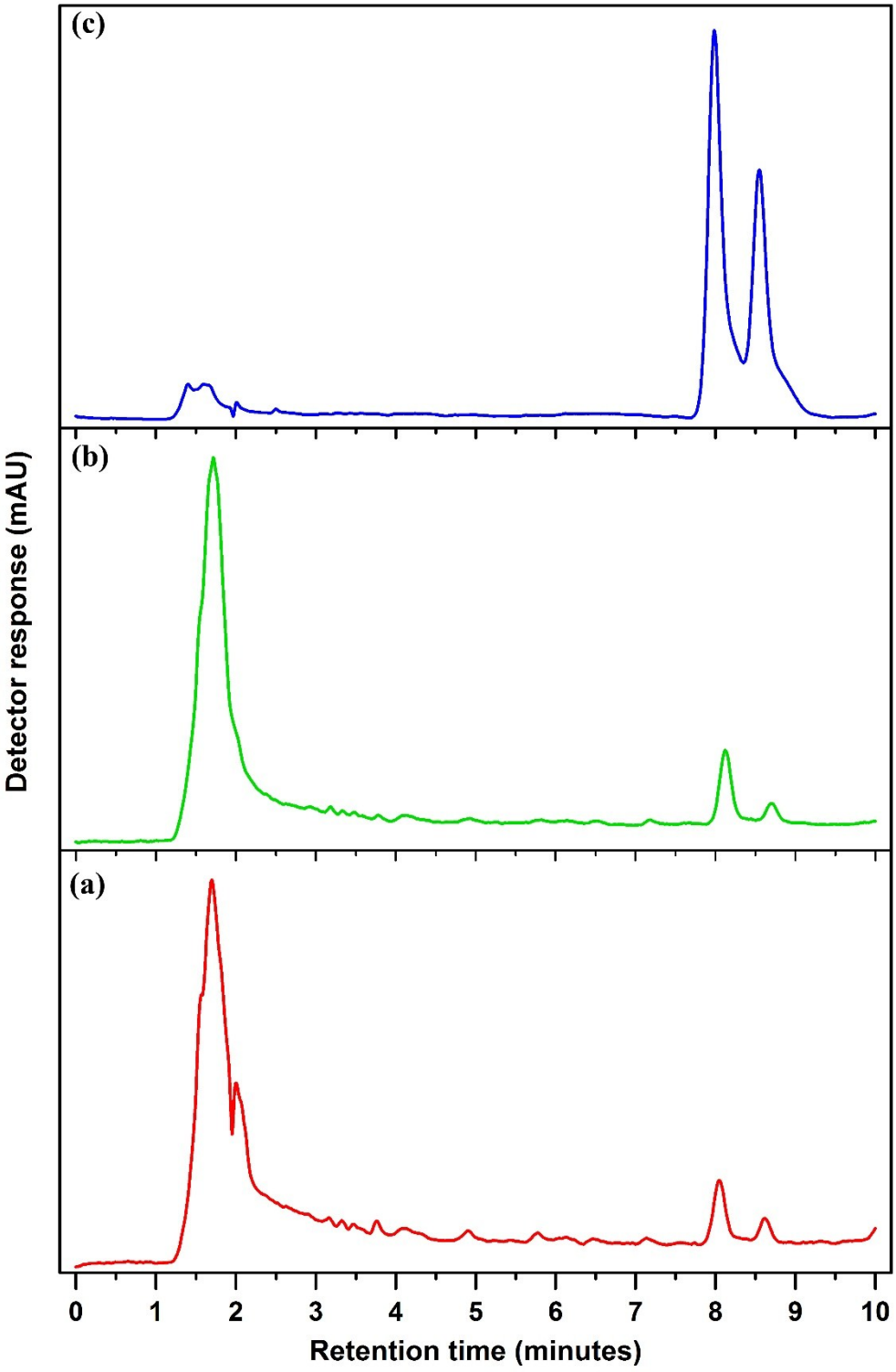

Figure S8. The chromatogram obtained by DMSPE-HPLC-UV for mentha under optimum conditions. (a) non-spiked, (b) spiked with 100 ng mL^{-1} before DMSPE and (c) extracted from Mentha sample after DMSPE of thymol and carvacrol.

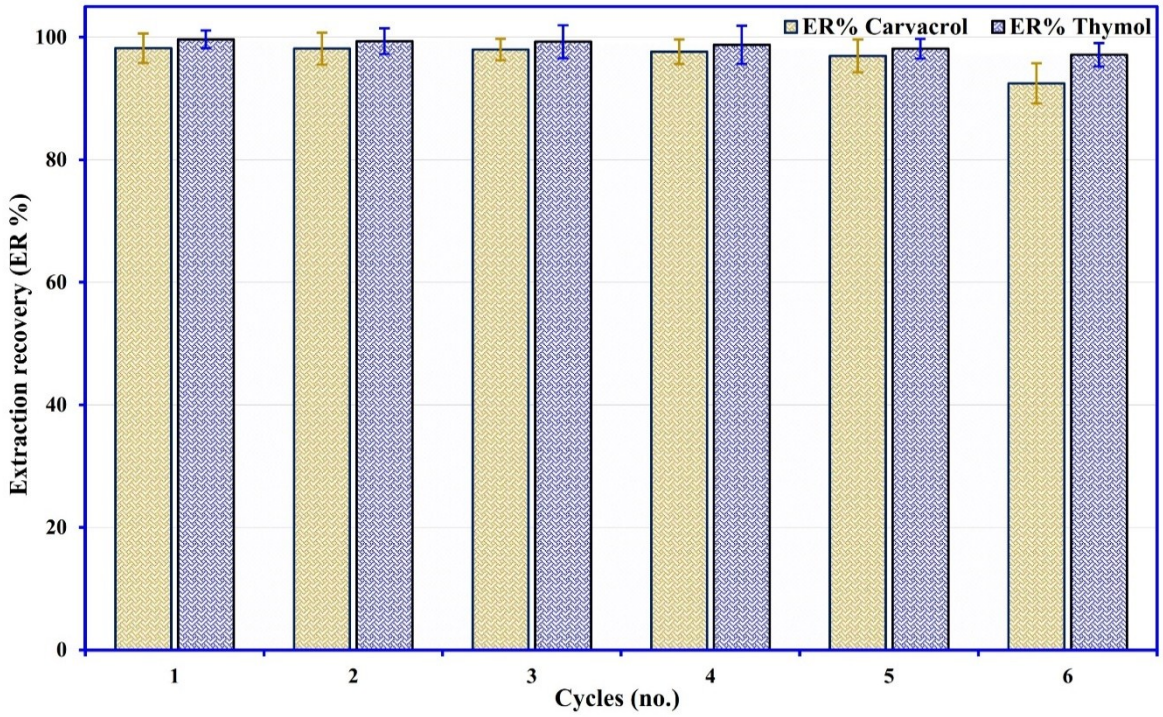

Figure S9. Reusability of nano-sorbent for DMSPE of thymol and carvacrol. The mean of three replicates was used as each extraction efficiency with error bar.

2. Tables

Table S1. CCD design matrix for five variables with the observed values for the recovery of thymol and carvacrol.

Independent variables	Unit	Levels (α =2)				
		-α	Low (-1)	Center (0)	High (+1)	+α
(X_1) pH	-	2.0	3.5	5.0	6.5	8.0
(X_2) Ionic strength (NaCl)	w/v%	0.00	0.25	0.50	0.75	1.00
(X_3) Nano-sorbent mass	mg	5.5	7.5	10.0	12.5	15.0
(X_4) Contact time	min	0.50	2.50	4.50	6.50	8.50
(X_5) Desorption volume	µL	50	125	200	275	350

Run	Factors					ER%	
	X_1	X_2	X_3	X_4	X_5	Carvacrol	Thymol
1	1	-1	-1	-1	-1	40.260	58.760
2	1	-1	-1	1	1	50.234	56.870
3	0	0	0	0	0	73.300	86.210
4	1	-1	1	-1	1	27.410	45.980
5	1	1	1	-1	-1	46.222	58.760
6	-2	0	0	0	0	84.150	94.760
7	0	0	0	0	0	74.040	83.110
8	0	0	-2	0	0	55.378	52.150
9	0	0	0	-2	0	39.676	63.708
10	1	1	1	1	1	84.117	97.980
11	0	0	0	0	0	69.180	82.270
12	-1	1	-1	1	1	80.442	76.434
13	-1	1	1	1	-1	60.517	78.970
14	0	0	2	0	0	81.235	82.400
15	-1	-1	1	-1	-1	60.060	69.870
16	0	0	0	0	0	69.140	82.560
17	0	-2	0	0	0	69.260	70.540
18	0	0	0	0	0	72.170	86.270
19	1	-1	1	1	-1	51.320	50.890
20	0	0	0	0	-2	45.430	54.652
21	1	1	-1	1	-1	33.422	45.518
22	0	2	0	0	0	63.800	59.043
23	-1	-1	-1	-1	1	64.270	85.890
24	-1	1	-1	-1	-1	63.876	59.359
25	-1	1	1	-1	1	86.508	91.469
26	0	0	0	0	0	71.150	85.410
27	1	1	-1	-1	1	31.855	30.873
28	-1	-1	1	1	1	98.706	99.870
29	0	0	0	0	2	70.119	76.040
30	2	0	0	0	0	33.376	48.150
31	-1	-1	-1	1	-1	64.885	73.650
32	0	0	0	2	0	68.418	80.143

Table S2. Analysis of variance (ANOVA) for the selected quadratic model.

		ER% Carvacrol				ER% Thymol			
Source	**Degree of freedom**	**Sum of squares**	**Mean square**	***F*-value**	***P*-value**	**Sum of squares**	**Mean square**	***F*-value**	***P*-value**
Model	20	9827.65	491.38	59.69	< 0.0001	9334	466.7	43.66	< 0.0001
X_1	1	4159.92	4159.92	505.32	< 0.0001	3339	3339	312.4	< 0.0001
X_2	1	14.87	14.87	1.81	0.2060	26.91	26.91	2.517	0.1409
X_3	1	785.79	785.79	95.45	< 0.0001	1161	1161	108.6	< 0.0001
X_4	1	1075.55	1075.55	130.65	< 0.0001	523.5	523.5	48.98	< 0.0001
X_5	1	967.22	967.22	117.49	< 0.0001	730	730	68.3	< 0.0001
X_1X_2	1	32.98	32.98	4.01	0.0706	119.2	119.2	11.16	0.0066
X_1X_3	1	27.51	27.51	3.34	0.0948	17.52	17.52	1.639	0.2268
X_1X_4	1	118.32	118.32	14.37	0.0030	74.6	74.6	6.979	0.0229
X_1X_5	1	211.66	211.66	25.71	0.0004	182.5	182.5	17.07	0.0017
X_2X_3	1	155.76	155.76	18.92	0.0012	954.1	954.1	89.26	< 0.0001
X_2X_4	1	116.14	116.14	14.11	0.0032	88.65	88.65	8.293	0.0150
X_2X_5	1	187.63	187.63	22.79	0.0006	21.88	21.88	2.047	0.1803
X_3X_4	1	130.74	130.74	15.88	0.0021	121.2	121.2	11.34	0.0063
X_3X_5	1	184.02	184.02	22.35	0.0006	256.2	256.2	23.97	0.0005
X_4X_5	1	672.51	672.51	81.69	< 0.0001	348.4	348.4	32.59	0.0001
X_1^2	1	279.69	279.69	33.97	0.0001	196.1	196.1	18.34	0.0013
X_2^2	1	38.53	38.53	4.68	0.0534	530.1	530.1	49.59	< 0.0001
X_3^2	1	14.46	14.46	1.76	0.2120	386.6	386.6	36.17	< 0.0001
X_4^2	1	534.04	534.04	64.87	< 0.0001	178.6	178.6	16.71	0.0018
X_5^2	1	326.25	326.25	39.63	< 0.0001	496.1	496.1	46.41	< 0.0001
Residual	11	90.55	8.23			117.6	10.69		
Lack of Fit	6	69.34	11.56	2.72	0.1456	100.3	16.71	4.822	0.05261
Pure Error	5	21.21	4.24			17.33	3.465		
Corr. Total	31	9918.21				9452			

Table S3. Characteristic data of the developed DMSPE-HPLC-UV method for the determination of thymol and carvacrol from methanolic extracts of plants and water samples.

Quantitative analysis	**Values**
Sample volume (mL)	15.0
extraction solvent (mL)	0.300
Linear range (ng mL^{-1})	0.4-6000
correlation coefficients (R^2)	0.985-0.999
Limit of detections (LODs) (ng mL^{-1})	0.054-0.104
Limit of quantification (LOQs) (ng mL^{-1})	0.178-0.345
Enrichment factor (EF)	100.5-222.8
Preconcentration factor (PF)	50.0
Reproducibility (RSD, %)	1.62-7.80
Repeatability (RSD, %)	1.37-5.20