Micelle assisted thin-film solid phase microextraction: a novel approach for determination of quaternary ammonium compounds in environmental samples

*Ezel Boyacı and Janusz Pawliszyn**

Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, N2L 3G1, Canada

*Corresponding author. Tel.: +1-519-888-4641; Fax: +1-519-746-0435

E-mail address: janusz@uwaterloo.ca

Table of Contents

1.1. Chemicals and solutions

The QAC standards decyltrimethylammonium bromide (DTMAB), trimethyltetradecyl ammonium chloride (TMTDAC), dodecyltrimethylammonium chloride (DDTAC), hexadecyltrimethylammonium chloride (HDTMAC), benzyldimethyldodecylammonium chloride (BAC12) and trimethyloctylammonium chloride (TMOAC) were purchased from Aldrich (Oakville, ON, Canada). Benzyldimethyltetradecylammonium chloride (BAC14) was purchased from Fluka (Oakville, ON, Canada). Benzyldimethylhexadecylammonium chloride (BAC16) and trimethyloctadecylammonium chloride (TMODAC) were obtained from Sigma (Oakville, ON, Canada). Internal standards (IS) decyl-d21-trimethylammonium bromide (DTMAB-d21) and n-hexadecyltrimethyl-d9-ammonium bromide (HDTMAB-d9) were obtained from CDN isotopes (Pointe-Claire, QC, Canada). Mobile phases and desorption solutions were prepared from LC grade acetonitrile, methanol, isopropyl alcohol and 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS hydrate), which were all obtained from Sigma Aldrich. LC-MS grade ammonium acetate and formic acid were supplied by Fluka (Oakville, ON, Canada).

Thin film SPME (TF-SPME) blades were prepared using the spraying method described by Mirnaghi et al[1]. For preparation of thin film 96-blade SPME coatings, hydrophilic lipophilic balance (HLB) SPE particles (polymeric reversed-phase, 60 µm particle size) were kindly obtained from Supelco. Polyacrylonitrile (PAN), obtained from Aldrich (Oakville, ON, Canada), was dissolved in N,N-dimethylformamide (Sigma-Aldrich, Oakville, ON, Canada) and used as glue for the immobilization of functional polymeric particles to the surface of blades. Nunc U96 Deep Well 2 mL plates made of polypropylene were purchased from VWR International (ON, Canada) and used in TF-SPME protocol.

1.2. Stock and working solutions

Standard and internal standard stock solutions were prepared at a 1.0 mg mL^{-1} concentration in methanol, and lower concentrations of mixed analytes stock solutions were prepared weekly. Working (extraction) solutions were prepared in ultrapure water or in ultrapure water containing an appropriate amount of CHAPS; this was accomplished by keeping the final amount of organic solvent at always less than 1% (v/v) in the final sample. All standard solutions were stored at 4 ^{o}C until required.

1.3. Instrumentation and chromatographic conditions

A Thermo TSQ Vantage (triple quadrupole) equipped with a heated electrospray ionization (H-ESI) ion source was used in positive mode and operated under selected reaction monitoring (SRM) conditions. Spray voltage was 1.0 kV; vaporizer and transfer capillary temperatures were 275 °C. Sheath and auxiliary gas (N_2) pressures were 40 AU and 15 AU, respectively. Argon was used as a collision gas, and pressure was kept at 1.5 mTorr. Optimized tuning parameters for each compound are shown in Table 1-S. The liquid chromatography system consisted of a Thermo Accela Pump equipped with an on-line vacuum degasser and a Thermo Accela Autosampler.

For the separation of analytes, A Gemini-NX phase 3µm C18 110A 50mm x2.00mm column with a Security Guard cartridge, Gemini-NX C18, 4x2.0 mm ID (Phenomenex) was used at early stage of the study and later replaced by a Discovery 3µm HS F5-5 phase 50 mm x2.1 mm ID column (Supelco). Chromatographic separation was achieved using solvent A: 10.0 mM ammonium acetate in acetonitrile/water (70/30, v/v) and solvent B: 0.1%, v/v formic acid in isopropyl alcohol at 500 µL min^{-1} flow rate. Gradient started at 90% A and 10% B and kept for 2.2 min at this composition, changed to 50% A and 50% B in 2 min and held for 1.9 min. Next, gradient

composition was changed to initial condition (90% A and 10% B) in 1 min, and then held at this condition for 1 min before next injection. For analysis, 5.0 µL of each samples were injected in partial loop mode. Cleaning the injection system after each injection was accomplished by flushing and washing (1 mL each) the syringe with a mixture of A:B (90/10, v/v), where A is 10.0 mM of ammonium acetate in 95/5, v/v, acetonitrile/ H_2O and B is 0.1% v/v formic acid in isopropyl alcohol.

Chromatographic data acquisition, peak integration, and quantification were performed using Xcalibur software v.2.0.7 (Thermo Fisher Scientific, San Jose, USA). Mobile phases were filtered through 0.45 µm nylon filter paper (Oakville, ON, Canada) and degassed for 20 min in a VWR Scientific Aquasonic model 75HT (West Chester, PA, USA) ultrasonic bath before being used. TF-SPME was performed using a manual Concept 96 kit (Professional Analytical System (PAS) Technology, Magdala, Germany).

1.4. Thin film SPME of QACs

The same extraction/desorption parameters reported by Boyaci et al. for extraction of QACs in TF-SPME (with hydrophilic lipophilic balance particles coated blades) using a manual Concept 96 kit were used in this study[2]. Any modifications in the applied conditions were stated in the related part of the text. Extractions were performed from 1.2 mL ultrapure and CHAPS-containing water spiked with analytes. Desorption was achieved in 1.2 mL of desorption solution, which is a mixture of A:B (70/30, v/v) acidified to contain 1% v/v formic acid, where A is 10.0 mM of ammonium acetate in 95/5, v/v, acetonitrile/ H_2O and B is isopropyl alcohol.

DTMAB-d21 and HDTMAB-d9 were used as internal standards by spiking the samples at a concentration of 20.0 ng mL^{-1}, in order to correct for variations in the sample preparation step, as

well as for instrumental and injection variations. Each step in the TF-SPME procedure described above was carried out with agitation at 850 rpm.

Calibration standard solutions for LC-MS/MS were prepared in the desorption solution in a range of 0.1 to 100.0 ng mL^{-1}, and used for calculation of the absolute amount of extracted analytes. External SPME calibration was used for real sample analysis. Quality control (QC) consisting of 25.0 ng mL^{-1} of each analyte was applied throughout the sequence to monitor the stability of the instrumental response.

1.5. Evaluation of CHAPS for adsorption losses correction

The difficulties associated with the determination of QACs in a laboratory settings have been already addressed in a former study[2]. In this study, the main purpose was to develop a novel SPME method for fast high-throughput laboratory analysis of QACs that is free of secondary interactions, and is easily applicable for routine analysis with minimum sample handling. For this purpose, the addition of a matrix modifier to the sample for elimination of secondary interactions between analytes and lab-wares was selected as the best approach. The desired properties for the most convenient matrix modifier for this study can be summarized as follows: minimum or no interaction with the extraction phase of SPME, no retention in applied chromatographic method (elution in void volume), low or no ion suppression in the MS analysis, effective for stabilization of all tested analytes in the matrix, and complete elimination of the secondary interactions.

After a scrutinized evaluation of the physicochemical properties of several candidates, CHAPS, a zwitterionic detergent, was chosen as a matrix modifier in view of the fact that it bears many of the abovementioned features. Owing to the moderately high polarity of CHAPS, it is expected that the addition of CHAPS should yield a minimal interaction with the SPME phase and the

chromatographic stationary phase. Moreover, its structural similarity to analytes (Figure 1-S) presumes that CHAPS may provide saturation in the active sites of the utilized lab-wares. As such, studies were conducted to find out the optimum concentration of matrix modifier, the most suitable point for addition of the matrix modifier in the sampling/sample preparation steps, as well as evaluation of a possible matrix effect on the analytical instrument and SPME.

1.5.1. Preliminary evaluation of the effect of CHAPS in external SPME calibrations

A preliminary study was performed in the presence of 1.0 mM and 3.0 mM CHAPS in extraction solutions while analytes concentrations were varying in a range of 5.0 to 1000.0 ng mL^{-1}. In addition, this evaluation was performed in two separate sets of experiments to find out the best approach for adding the matrix modifier into the sample, namely, post-addition and pre-addition of CHAPS into the sample vial.

In the post-addition approach, analytes were spiked into ultrapure water collected in a 20 mL vial. To ensure equilibration, vials were agitated for 60 min at 1200 rpm; this step also promoted any possible adsorption losses to the walls of the vials. Next, an appropriate amount starting from 10.0 mM CHAPS solution was spiked into the samples to get a 1.0 mM of CHAPS in the final solutions. Then, samples were equilibrated for 60 min by shaking and transferred to the 96-well plate for TF-SPME. For the pre-addition approach, analytes were spiked into a vial containing 1.0 mM CHAPS solution and equilibrated for 60 min by agitation at 1200 rpm; next, an appropriate amount of sample was transferred into the 96-well plate for TF-SPME.

1.5.2. Matrix effect in presence of CHAPS

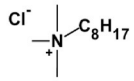
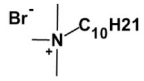
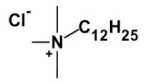
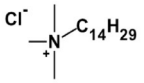
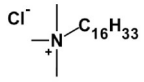
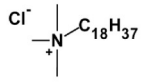
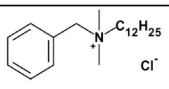
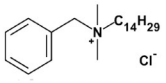
Matrix effects in presence of CHAPS were evaluated both for SPME and LC-MS/MS. The absolute matrix effect in presence of CHAPS in the sample (ion suppression or enhancement in

LC-MS/MS) was investigated at 25.0 ng mL^{-1} analytes concentration. For this purpose, extraction from 1.2 mL of 1.0 mM CHAPS solution was performed to obtain the extract free of target analytes, and then this extract was spiked with QACs in order to get a final concentration of 25.0 ng mL^{-1} of each target analyte. The obtained peak areas were compared with the peak areas of target analytes spiked in the neat desorption solution at the same concentration level as the extracts described. A possible matrix effect on SPME was also evaluated at two levels of concentrations (25.0 and 250.0 ng mL^{-1}). According to the obtained external SPME calibration curves, the effect of secondary interaction of the compounds is more pronounced at low concentration levels (including analyte concentration of 25.0 ng mL^{-1}) where the saturation of active sites on lab-ware is not achieved and the amount of analye available for extraction is substantially lower than the spiked amount. As concentration of analyte increases a positive deviation from linearity is observed (for concentrations higher than 100.0 ng mL^{-1}) which indicates that the active sites are saturated and the available amount of analyte in sample for extraction is higher. Thus, 25.0 and 250.0 ng mL^{-1} were selected as representative concentrations of analytes that allows the matrix effect investigation to be performed in more realistic way. For this purpose, two TF-SPME experiments were performed; the first extraction was from ultrapure water spiked with analytes, and the second extraction was performed from 1.0 mM of CHAPS solution spiked with analytes. The ratio of amount of extracted analytes from ultrapure water to extracted analytes from CHAPS solution was used to evaluate the matrix effect on SPME.

According to a preliminary evaluation, for the first two eluting compounds (close to void volume), significant absolute matrix effects were observed. In order to eliminate the absolute matrix effect, the C18 column (Phenomenex, 3µm Gemini-NX phase, 50 mm x2.0 mm ID) was replaced with a HS-F5 bonded fluoro (pentafluorophenyl) column (Supelco, 3µm HS F5-5 phase, 50 mm x2.1 mm

ID) so as to improve the retention of analytes. The two compounds eluting close to void volume in the C18 column (retention time: 0.32 min and 0.42 min) were retained better in the PFP column (retention time: 1.88 min and 2.70 min), while CHAPS was still eluting in the void volume. This strategy eliminated the absolute matrix effect completely in the study.

Table 1-S. Optimized tuning parameters and SRM transitions of compounds in Thermo TSQ Vantage

Compound name	Chemical structure	Parent mass m/z	Product mass m/z	Collision energy (V)	S Lens (V)
Trimethyloctylammonium chloride TMOAC		172.167	60.119	10	65
Decyltrimethylammonium bromide DTMAB		200.176	60.116	22	82
Dodecyltrimethylammonium chloride DDTAC		228.188	60.112	24	89
Trimethyl-teradecyl-ammonium chloride TMTDAC		256.197	60.108	27	94
Hexadecyltrimethylammonium chloride HDTMAC		284.197	60.107	29	108
Trimethyloctadecylammonium chloride TMODAC		312.156	60.098	30	115
Benzalconium chloride BAC12		304.292	212.237	19	101
Benzalconium chloride BAC14		332.317	240.259	21	116

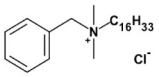
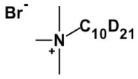
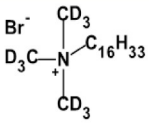
Benzalconium chloride BAC16		360.161	268.336	10	58
Decyl-d21-trimethylammonium bromide (IS) DTMAB-d21		221.360	61.200	25	120
n-hexadecyltrimethyl-d9-ammonium bromide (IS) HDTMAB-d9		294.172	69.221	31	123

Table 2-S. Matrix effect of 1 mM CHAPS at 25.0 ng mL^{-1} and 250.0 ng mL^{-1} analyte concentrations

Analyte		Absolute matrix effect (%)		Matrix effect on SPME (%)	
	logP	25 ng mL^{-1}	250 ng mL^{-1}	25 ng mL^{-1}	250 ng mL^{-1}
TMOAC	-1.05	90.4	86.5	97.4	97.7
DTMAB	-0.39	98.0	91.1	107.5	119.6
DDTAC	0.67	100.2	89.9	123.6	127.3
TMTDAC	1.25	99.2	91.1	208.6	123.3
BAC12	1.69	98.4	90.6	289.8	128.7
HDTMAC	2.06	98.5	91.7	141.4	112.6
BAC14	2.55	97.5	91.8	283.1	133.6
TMODAC	3.26	94.9	89.6	85.7	90.1
BAC16	3.42	97.4	88.0	80.5	164.5

Table 3-S. Amount of analytes remained in extraction plate as a function of CHAPS concentration

Analyte	logP	Amount of analytes remained in extraction plate (ng)				
		Water	0.1 mM CHAPS	1.0 mM CHAPS	10.0 mM CHAPS	50.0 mM CHAPS
TMOAC	-1.05	nd	nd	nd	nd	nd
DTMAB	-0.39	˂LOQ	˂LOQ	˂LOQ	˂LOQ	˂LOQ
DDTAC	0.67	0.46 (± 0.14)	˂LOQ	˂LOQ	nd	˂LOQ
TMTDAC	1.25	4.14 (± 0.94)	0.01 (± 0.06)	nd	nd	nd
BAC12	1.69	5.24 (± 1.75)	0.04 (± 0.06)	nd	nd	nd
HDTMAC	2.06	7.01 (± 1.88)	2.23 (± 0.46)	2.28 (± 1.51)	nd	nd
BAC14	2.55	5.76 (± 1.72)	2.48 (± 0.44)	0.76 (± 0.59)	0.43 (± 0.06)	0.52 (± 0.22)
TMODAC	3.26	8.30 (± 1.64)	5.14 (± 1.03)	6.14 (± 1.58)	0.12 (± 0.07)	0.14 (±0.01)
BAC16	3.42	8.45 (± 0.91)	3.53 (± 0.71)	3.70 (± 1.14)	nd	nd

nd: not detected

Table 4-S. Precision of the method (intra-day precision (%RSD), n=6 and inter-day precision (%RSD), n=3) in 10.0 mM CHAPS

	Intra-day precision			Inter-day precision		
Analyte	**5.0 ng mL^{-1}**	**25.0 ng mL^{-1}**	**250.0 ng mL^{-1}**	**5.0 ng mL^{-1}**	**25.0 ng mL^{-1}**	**250.0 ng mL^{-1}**
TMOAC	6.3	4.8	6.0	22.9	11.0	14.7
DTMAB	3.9	2.5	2.6	1.3	2.1	5.0
DDTAC	2.7	1.0	3.2	1.4	0.9	7.7
TMTDAC	4.9	2.0	2.5	2.7	3.4	2.5
BAC12	4.4	0.9	1.7	1.9	2.2	6.4
HDTMAC	5.4	1.4	1.5	1.7	2.3	0.7
BAC14	3.6	0.5	3.0	4.7	3.2	3.6
TMODAC	5.3	1.7	2.1	3.0	1.6	1.7
BAC16	10.8	4.9	5.3	8.2	5.7	16.3

Table 5-S. Accuracy of the method (n=3) in 10.0 mM CHAPS containing natural water

	Spiked amount								Figures of merit			
	0.50 ng mL^{-1}		5.00 ng mL^{-1}		25.00 ng mL^{-1}		60.00 ng mL^{-1}					
Analyte	**Obtained ng mL^{-1}**	**% Recovery**	**Obtained ng mL^{-1}**	**% Recovery**	**Obtained ng mL^{-1}**	**% Recovery**	**Obtained ng mL^{-1}**	**% Recovery**	**LOD ng mL^{-1}**	**LOQ ng mL^{-1}**	**Regression equation**	**R^2**
TMOAC[a]	0.51 (± 0.04)	101.2	4.74 (± 0.11)	94.7	26.24 (± 0.36)	104.9	64.51 (± 1.41)	107.5	0.02	0.07	y = 0.154x - 0.057	0.999
DTMAB[a]	0.51 (±0.02)	102.1	4.87 (± 0.15)	97.5	27.27 (± 0.74)	109.1	66.01 (± 1.33)	110.0	0.02	0.06	y = 0.192x - 0.084	0.999
DDTAC[a]	0.55 (± 0.02)	109.7	4.77 (± 0.18)	95.4	24.98 (± 0.61)	99.9	65.64 (± 1.93)	109.4	0.03	0.11	y = 0.191x - 0.054	0.999
TMTDAC[a]	0.49 (± 0.02)	98.1	4.73 (± 0.28)	94.6	23.66 (± 0.61)	94.6	64.45 (± 2.79)	107.4	0.07	0.24	y = 0.134x - 0.043	0.999
BAC12[a]	0.43 (± 0.04)	86.2	4.70 (± 0.59)	93.9	23.64 (± 0.56)	94.6	63.99 (± 3.39)	106.7	0.15	0.49	y = 0.804x - 0.110	0.998
HDTMAC[b]	0.49 (± 0.03)	98.0	4.49 (± 0.14)	89.8	26.90 (± 1.06)	107.6	63.94 (± 1.43)	106.6	0.07	0.24	y = 0.329x - 0.110	0.998
BAC14[b]	0.55 (± 0.09)	110.0	4.28 (± 0.10)	85.6	25.92 (± 0.83)	103.7	64.66 (± 2.22)	107.8	0.21	0.69	y = 0.968x + 1.194	0.998
TMODAC[b]	0.50 (± 0.07)	99.7	4.23 (± 0.14)	84.6	24.76 (± 0.68)	99.0	58.92 (± 2.16)	98.2	0.11	0.36	y = 0.463x - 0.042	0.998
BAC16[b]	0.43 (± 0.02)	86.2	4.12 (± 0.12)	82.4	23.52 (± 0.74)	94.1	60.10 (± 2.80)	100.2	0.11	0.38	y = 1.376x - 0.187	0.998

External SPME calibrations were performed in ultrapure water containing 10.0 mM CHAPS

[a] DTMAB-d21used as IS

[b]HDTMAB-d9 used as IS

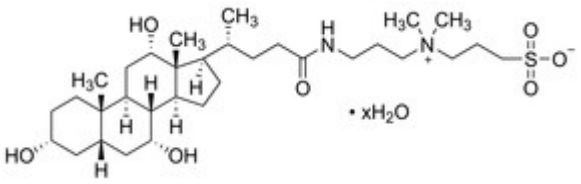
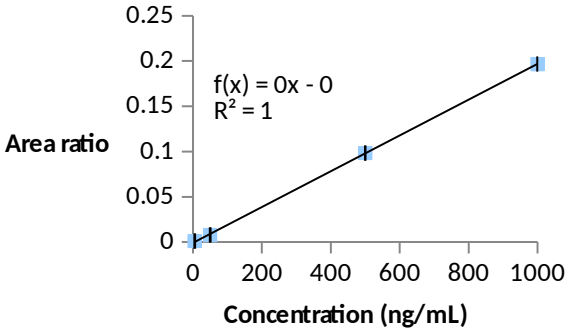
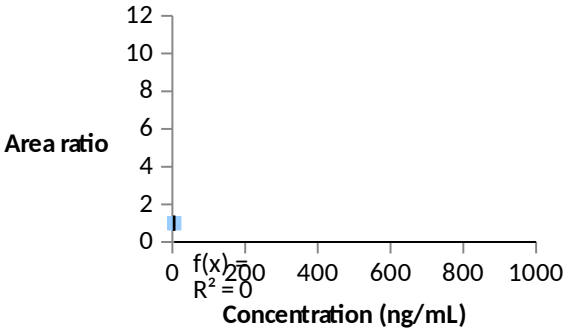


Figure 1-S. Structure of CHAPS

TMOAC (C8)

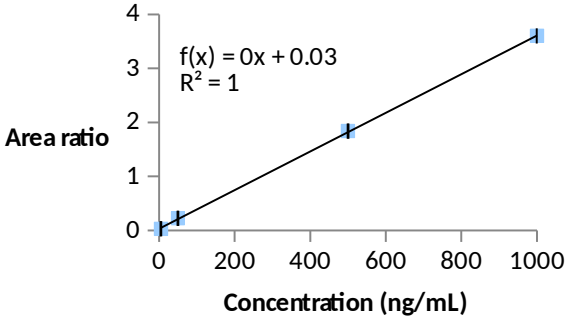


In 1 mM CHAPS

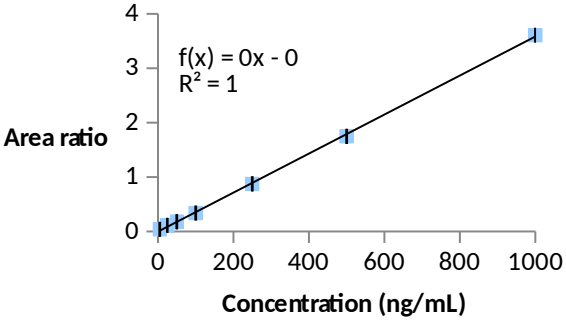


In 3 mM CHAPS

DTMAB (C12)

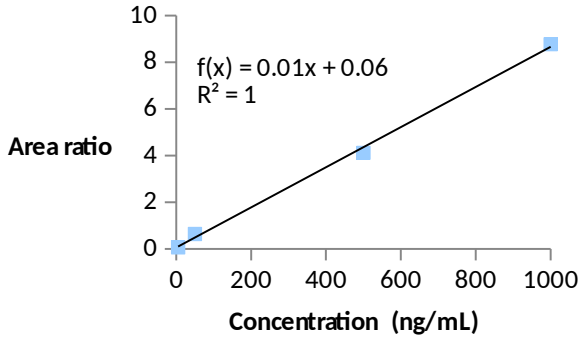


In 1 mM CHAPS

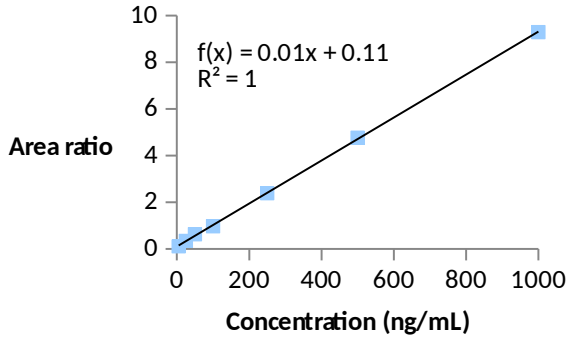


In 3 mM CHAPS

BAC12

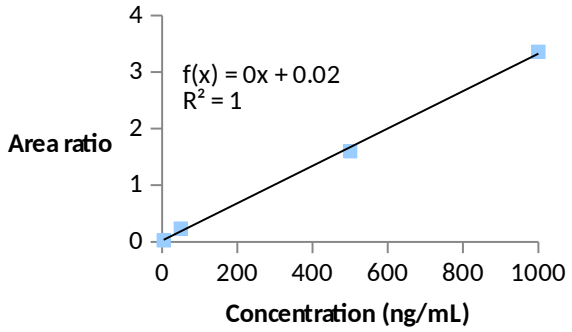


In 1 mM CHAPS

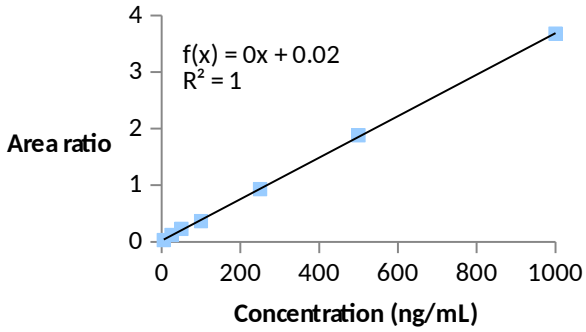


In 3 mM CHAPS

TMTDAC (C14)

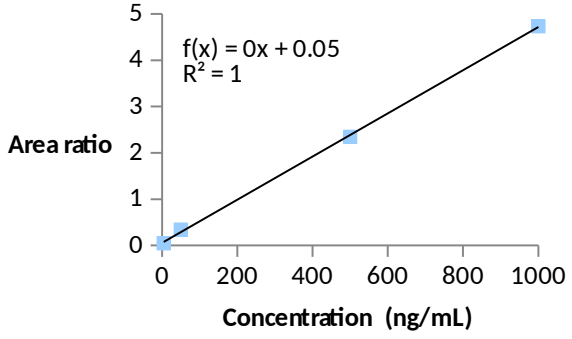


In 1 mM CHAPS

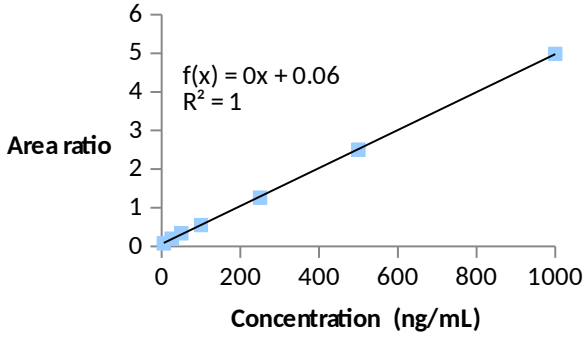


In 3 mM CHAPS

DDTAC (C12)

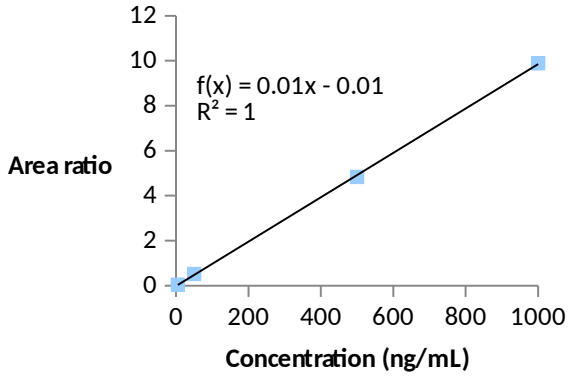


In 1 mM CHAPS

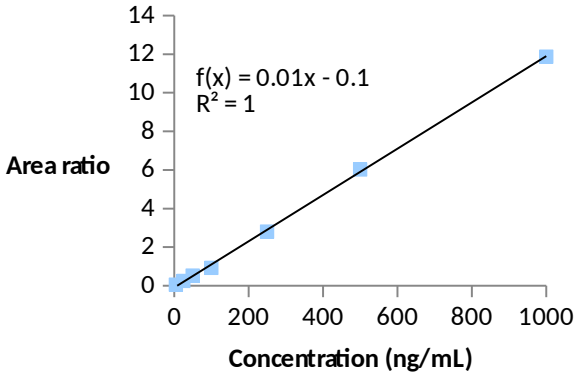


In 3 mM CHAPS

BAC14

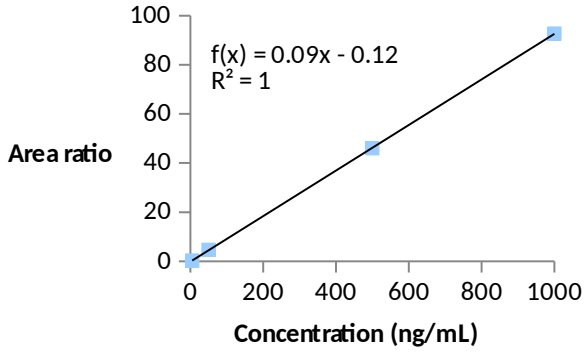


In 1 mM CHAPS

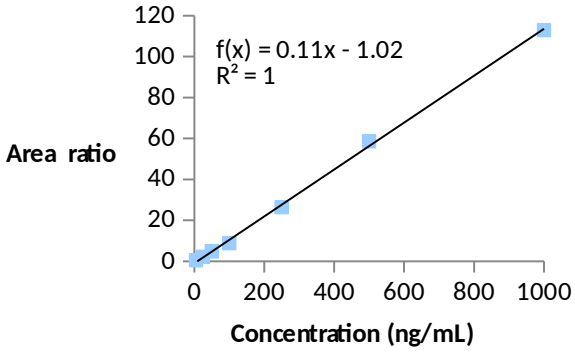


In 3 mM CHAPS

HDTMAC (C16)

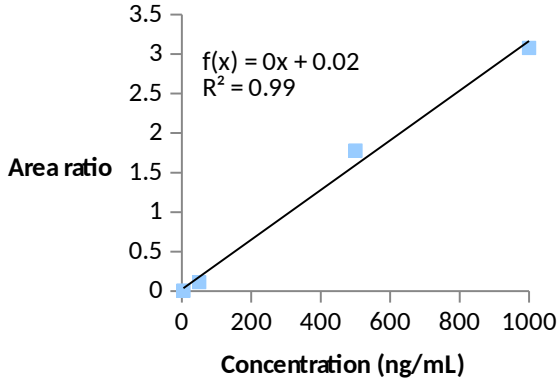


In 1 mM CHAPS

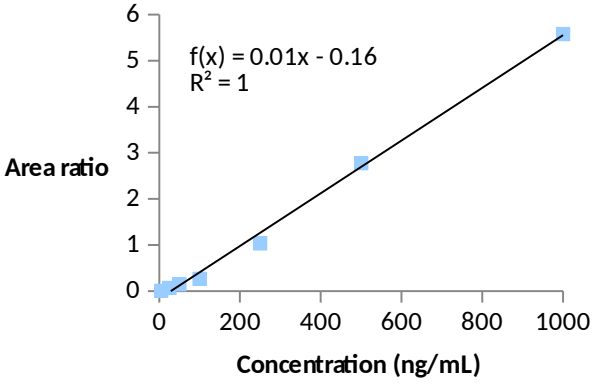


In 3 mM CHAPS

BAC16

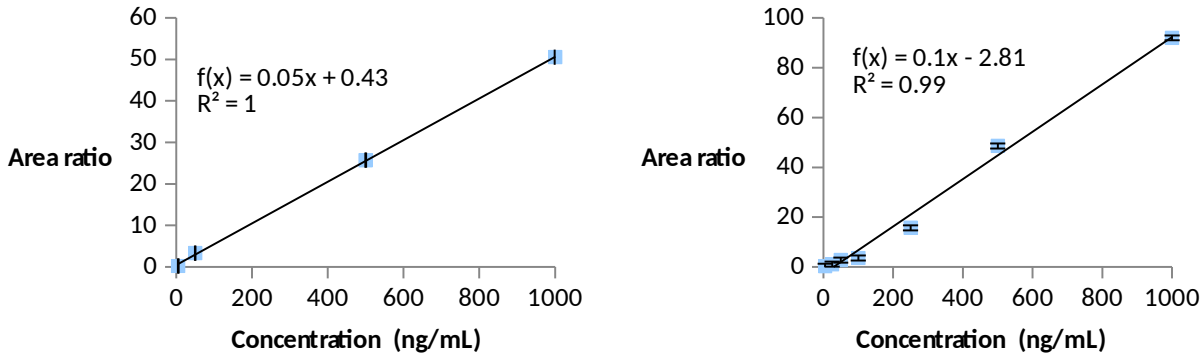


In 1 mM CHAPS



In 3 mM CHAPS

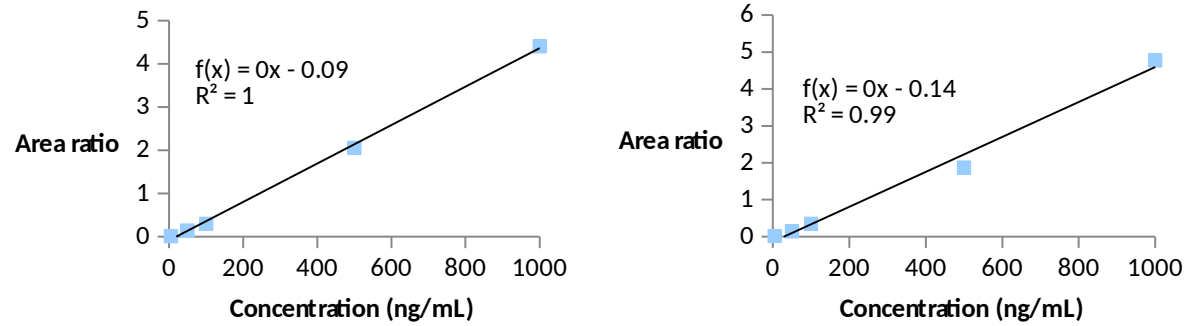
TMODAC (C18)



In 1 mM CHAPS In 3 mM CHAPS

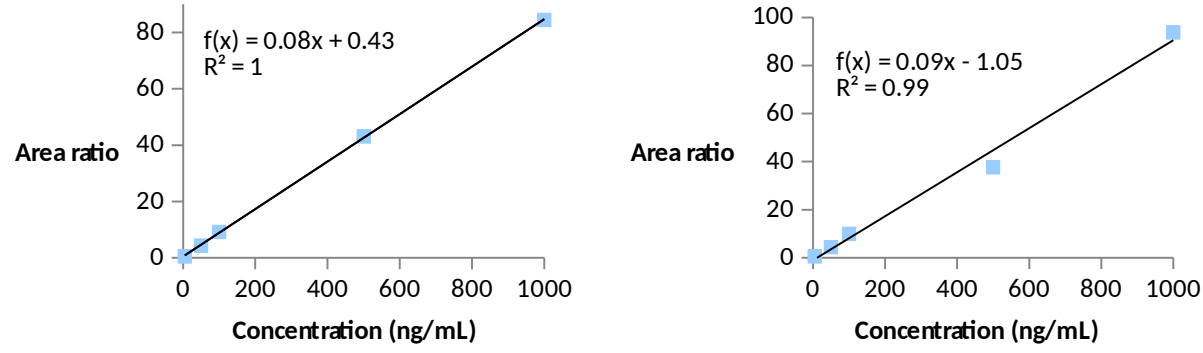
Figure 2-S. External SPME calibration obtained in presence of 1 mM and 3 mM CHAPS

TMOAC (C8)



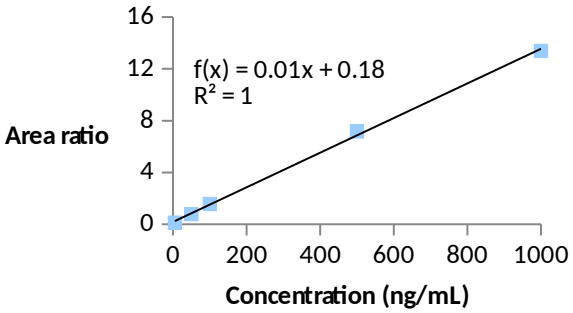
In 1 mM CHAPS (pre-spiked) In 1 mM CHAPS (post-spiked)

DTMAB (C12)

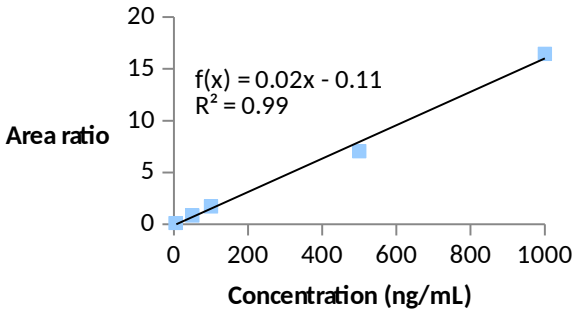


1 mM CHAPS (pre-spiked) In 1 mM CHAPS (post-spiked)

BAC12

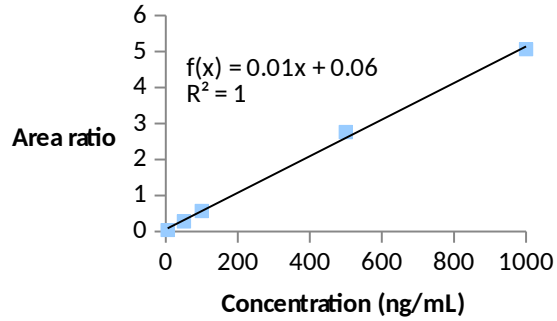


In 1 mM CHAPS (pre-spiked)

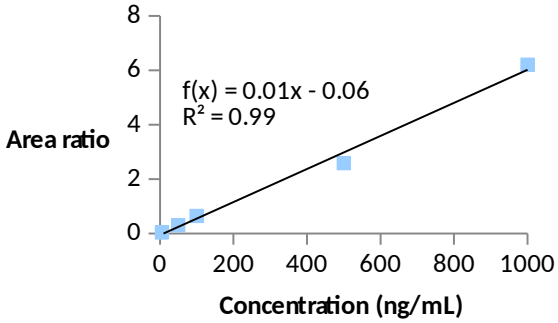


In 1 mM CHAPS (post-spiked)

TMTDAC (C14)

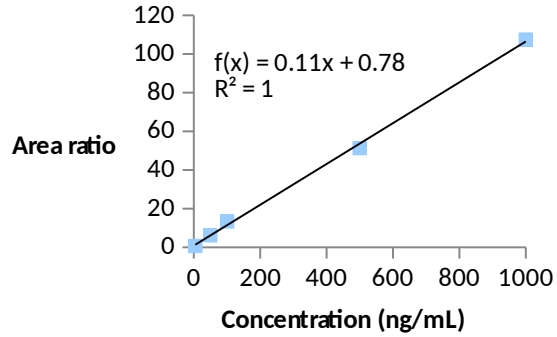


In 1 mM CHAPS (pre-spiked)

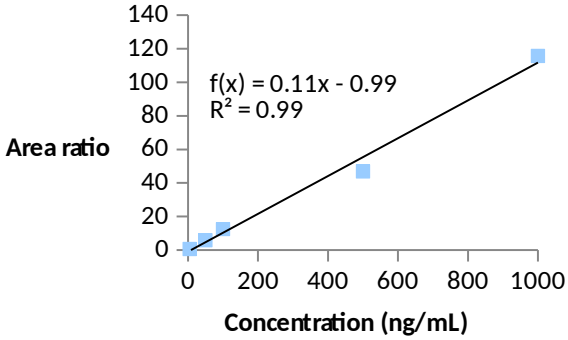


In 1 mM CHAPS (post-spiked)

DDTAC (C12)

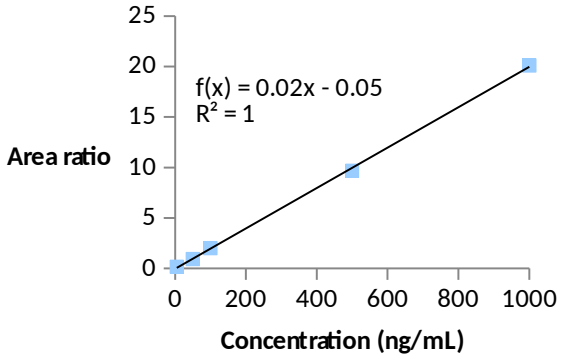


In 1 mM CHAPS (pre-spiked)

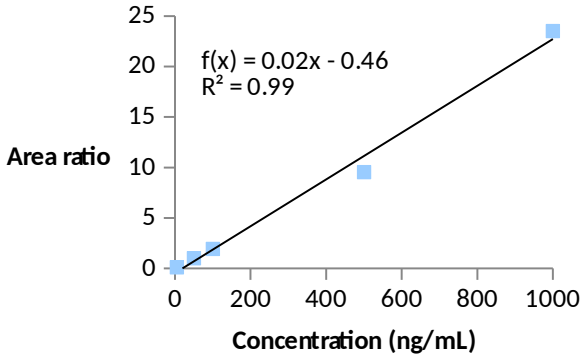


In 1 mM CHAPS (post-spiked)

BAC14

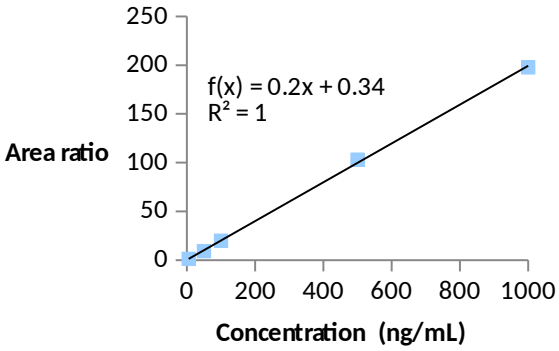


In 1 mM CHAPS (pre-spiked)

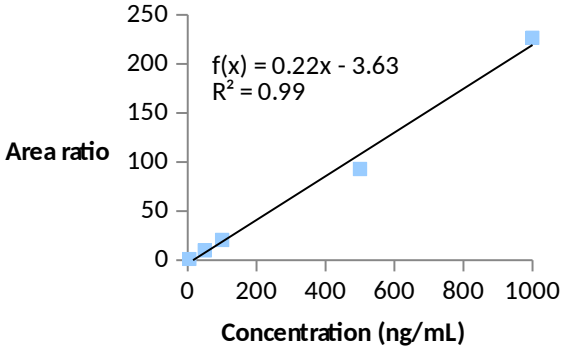


In 1 mM CHAPS (post-spiked)

HDTMAC (C16)

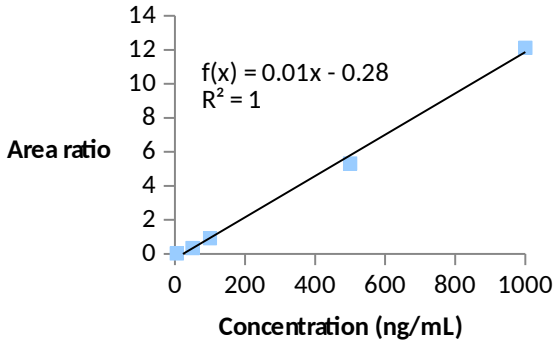


In 1 mM CHAPS (pre-spiked)

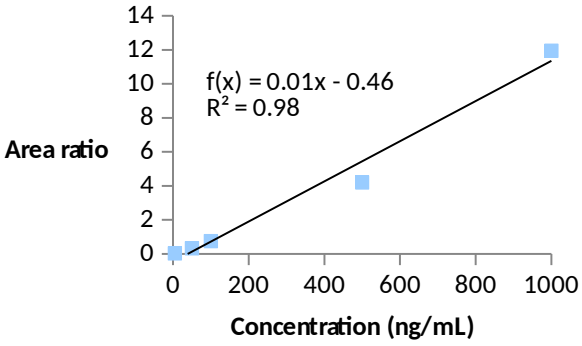


In 1 mM CHAPS (post-spiked)

BAC16

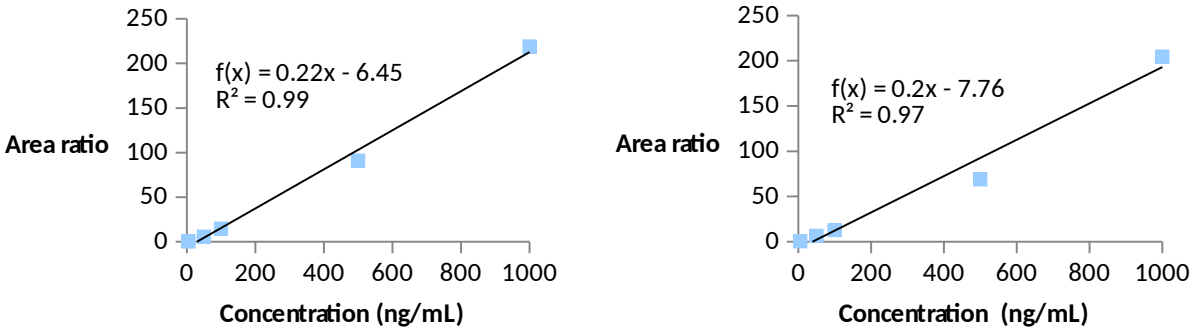


In 1 mM CHAPS (pre-spiked)



In 1 mM CHAPS (post-spiked)

TMODAC (C18)



In 1 mM CHAPS (pre-spiked) In 1 mM CHAPS (post-spiked)

Figure 3-S. External SPME calibration obtained in presence of 1 mM CHAPS, comparison of pre-spiking and post spiking of matrix modifier

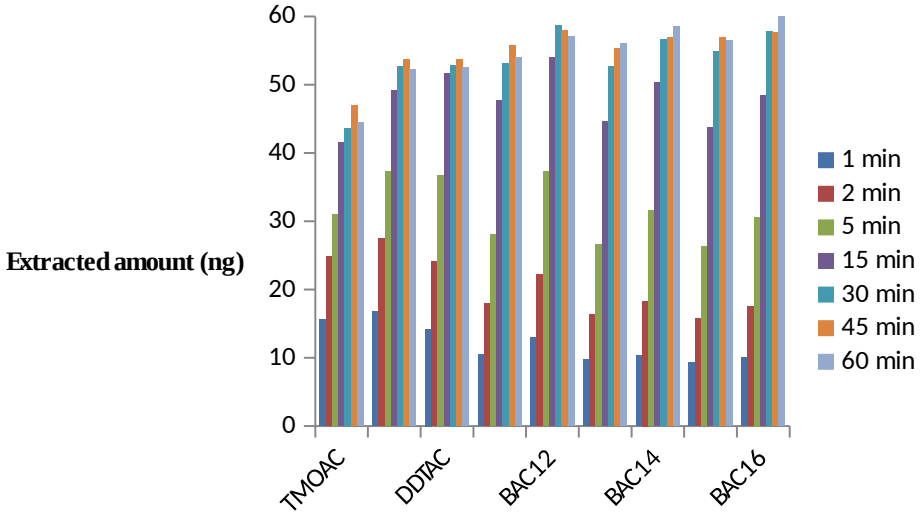
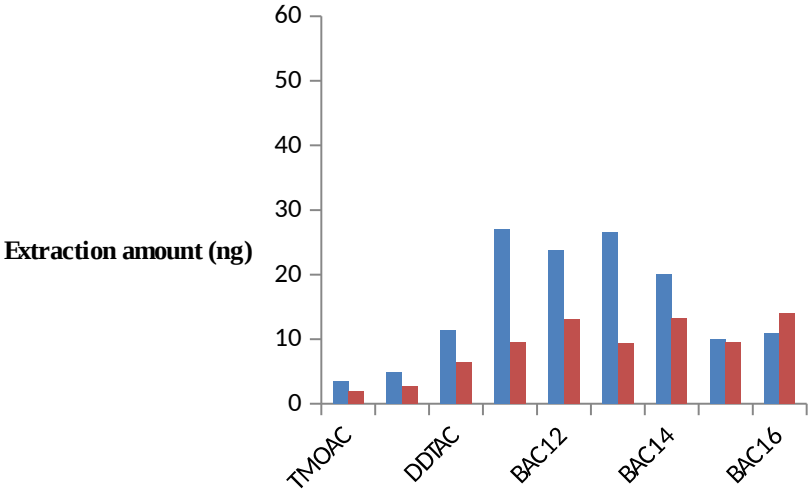
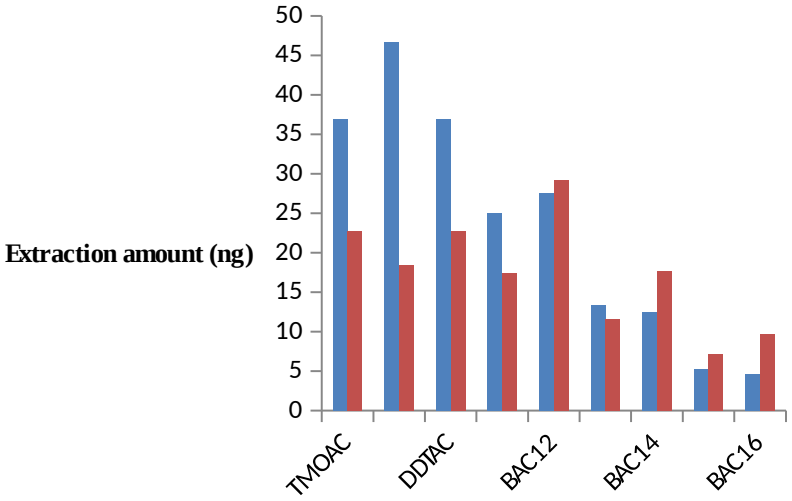


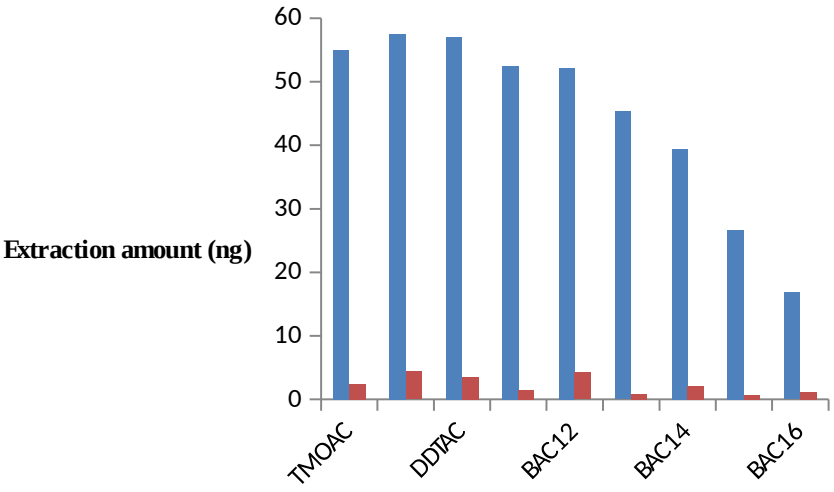
Figure 4-S. Extraction time profile of QACs by HLB coating in presence of 10.0 mM CHAPS


Extraction amount (ng)
60
50
40
30
20
10
0
TMOAC
DDTAC
BAC12
BAC14
BAC16

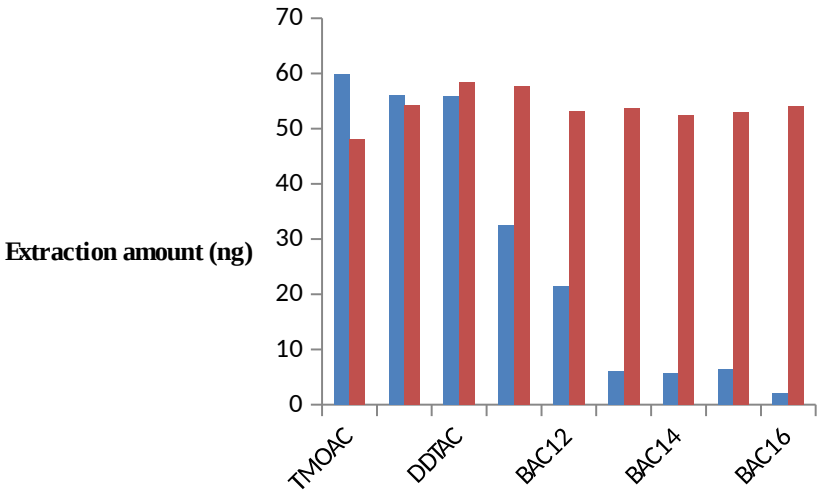
(a)


Extraction amount (ng)
50
45
40
35
30
25
20
15
10
5
0
TMOAC
DDTAC
BAC12
BAC14
BAC16

(b)



(c)



(d)

Figure 5-S. Extractions of QACs in absence of CHAPS (■) and in presence of 10 mM CHAPS in solution (■) with a) graphene, b) C18, c) PS-DVB-WAX and d) HLB coated TF-SPME. (Extraction conditions were; extraction volume: 1.2 mL, concentration of analytes: 50.0 ng mL^{-1}, extraction time: 45 min, desorption volume: 1.2 mL and desorption time: 15 min)

References

(1) Mirnaghi, F.S.; Chen, Y.; Sidisky, L.M.; Pawliszyn, J. Anal. Chem. 2011, 83, 6018–6025.

(2) Boyacı, E.; Sparham, C.; Pawliszyn, J. Anal. Bioanal. Chem. 2014, 406, 409–420.