Liquid chromatography with micelles in open-tube capillaries

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This Electronic Supplementary Information (ESI) pdf file includes:

Table S1. Relative retention time (t_R/t_0) of selected analytes in the proposed OT-LC.

pseudophase	CTAB t_R/t_0	SDS t_R/t_0		CTAB t_R/t_0	SDS t_R/t_0
cations			*anions*		
nicardipine	1.71	-	flufenamic acid	2.51	1.11
labetalol	1.63	1.39	mefenamic acid	2.30	1.06
imipramine	1.57	-	4-bromophenol	1.96	1.50
verapamil	1.54	-	fenbufen	1.82	-
dibucaine	1.49	-	fenoprop	1.79	-
propranolol	1.40	-	ibuprofen	1.72	-
naphthylamine	1.26	1.51	dichlorprop	1.63	-
diphenhydramine	1.23	-	4-vinylbenzene-sulfonic acid	1.63	-
alprenolol	1.17	2.84	sulindac	1.57	-
chlorpheniramine	1.14	2.73	4-nitrophenol	1.56	-
3-hydroxypyridine	1.06	-	furosemide	1.31	-
pindolol	1.04	1.47	chloramphenicol	1.09	1.06
nadolol	1.04	1.07	4-methoxyphenol	1.08	1.04
dibenzoquat	-	4.02			
diquat	-	1.16	*amphiphilic*		
neostigmine	-	1.04	sulfaquinoxaline	1.74	1.08
			sulfamethizole	1.60	-
p-nitroaniline	-	1.17	sulfadimethoxine	1.55	-
p-toluidine	-	1.04	sulfamethoxazole	1.39	-
ranitidine	-	1.03	sulfamerazine	1.12	-
neutrals			*amino acids/peptides/protein*		
chlorpyriphos	2.16	-	tyr-tyr-tyr	1.74	-
biphenyl	1.70	-	ubiquitin	1.44	-
acenaphthene	1.64	-	glu-val-phe	1.43	1.02
parathion	1.59	2.76	tryptophan	1.23	-
fenitrothion	1.52	2.13	phenylalanine	1.05	-
diazinon	1.46	1.66	bradykinin	-	1.27
prednisolone	1.40	1.08			
hydrocortisone	1.33	1.11			
azinphos-methyl	1.32	2.06			

Mobile phase was 1 mM CTAB or 1 mM SDS in 100 mM ammonium bicarbonate pH 8.5. Capillary was 50 μm i.d. and was unmodified (CTAB) or modified (SDS) with a cationic polyelectrolyte poly(diallyldimethylammonium chloride). Injection length was 2 mm. Separation was by pressure at a flow rate of 10 cm/min. Detection was at 200 nm. More analytes were retained with CTAB as pseudophase.

Table S2. Analytical figures of merit for the OT-LC with CTAB of pesticides.

	analyte				
	atrazine	diuron	diazinon	fenitrothion	parathion
Linearity					
concentration range, *x 10^{-4}* M	1.2 – 9.3	1.1 – 8.6	1.5 – 12	1.8 – 14	2.1 – 17
equation of line (y = mx+b)					
slope (m)	14.42	19.31	1.41	1.30	0.50
y-intercept (b)	+ 99.28	+ 9.24	+ 43.76	+ 73.61	+ 66.02
coefficient of variation (R^2)	0.996	0.998	0.996	0.998	0.999
limit of detection (S/N = 3), *x 10^{-4}* M	0.06	0.07	1.30	0.89	1.30
Repeatability, RSD (%)					
retention time					
intraday (n=3)[1]	0.2 – 0.7	0.1 – 1.1	0.05 – 0.8	0.2 – 0.9	0.3 – 2.0
interday (n=15)[2]	0.8	1.2	2.8	3.2	3.0
peak area					
intraday (n=3)[1]	2.8 – 5.5	1.0 – 5.5	0.2 – 4.2	1.6 – 4.4	0.4 – 6.0
interday (n=15)[2]	5.6	5.4	4.5	4.9	7.6

[1]RSD% range taken within 5 days with three replicates (n=3) per day.

[2]Taken from 15 pooled peak areas (n=15) within 5 days.

Table S3. Analytical figures of merit for the OT-LC with SDS of antioxidants.

	analyte				
	propyl gallate	butylated hydroxy-toluene	*tert*-butyl-hydroquinone	butylated hydroxy-anisole	parathion
Linearity					
concentration range, *x 10*$^{-4}$ M	1.1 – 8.8	1.4 – 11	2.3 – 18	1.7 – 14	1.4 – 12
equation of line (y = mx+b)					
slope (m)	14.6	7.65	4.25	5.71	1.49
y-intercept (b)	+ 241.49	- 19.44	- 56.83	- 26.23	- 3.36
coefficient of variation (R^2)	0.996	1.000	0.998	0.992	0.996
limit of detection (S/N = 3), *x 10*$^{-4}$ M	0.12	0.31	1.20	0.77	0.58
Repeatability, RSD (%)					
retention time					
intraday (n=3)[1]	0.1 – 2.0	0.2 – 2.1	0.2 – 1.2	0.2 – 3.1	0.3 – 1.9
interday (n=15)[2]	0.7	0.9	1.1	2.8	2.8
peak area					
intraday (n=3)[1]	1.0 – 8.4	1.9 – 5.6	1.0 – 7.3	0.4 – 3.4	0.8 – 9.2
interday (n=15)[2]	5.5	4.7	11.6	5.2	10.1

[1]RSD% range taken within 5 days with three replicates (n=3) per day.120

[2]Taken from 15 pooled peak areas (n=15) within 5 days.

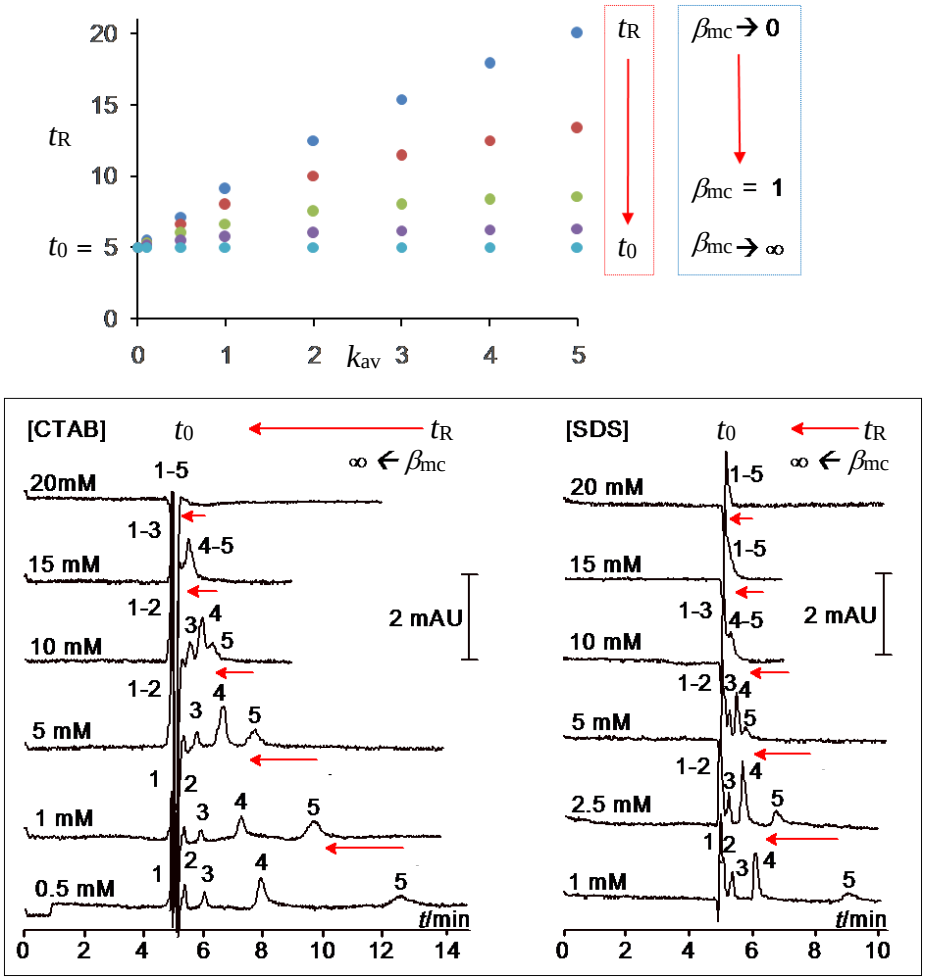

Figure S1. (top) The plot of analyte's k_{av} from 0.1-5 versus calculated t_R. The $t_R = t_0$ if the analyte was not retained or solubilised into the interfacial micelles. The increase in the micellar phase ratio or $\beta_{mc} \rightarrow \infty$ caused the $t_R \rightarrow t_0$. At $\beta_{mc} = 1$, the micelle solubilised analytes were equally distributed to the interfacial and solution micelles. (bottom) Experimental verification of the trend of $t_R \rightarrow t_0$ as $\beta_{mc} \rightarrow \infty$, using 25 µm i.d. capillaries. The $\beta_{mc} \rightarrow \infty$ when the CTAB concentration [CTAB] or SDS concentration [SDS] in 100 mM ammonium bicarbonate pH 8.5 as mobile phase was increased from 0.5 mM CTAB or 1 mM SDS to 20 mM. The analytes were mostly solubilised in the solution micelles with the 20 mM CTAB or SDS in the mobile phase. The analytes were acetophenone (peak 1), propiophenone (peak 2), butyrophenone (peak 3), valerophenone (peak 4) and hexanophenone (peak 5), in the order of increasing hydrophobicity or k_{av}. The analyte concentrations were 0.4-1.1 mM and injected sample plug was 2 mm. The mobile phases were flowed at a rate of 10 cm/min (180 mbar).

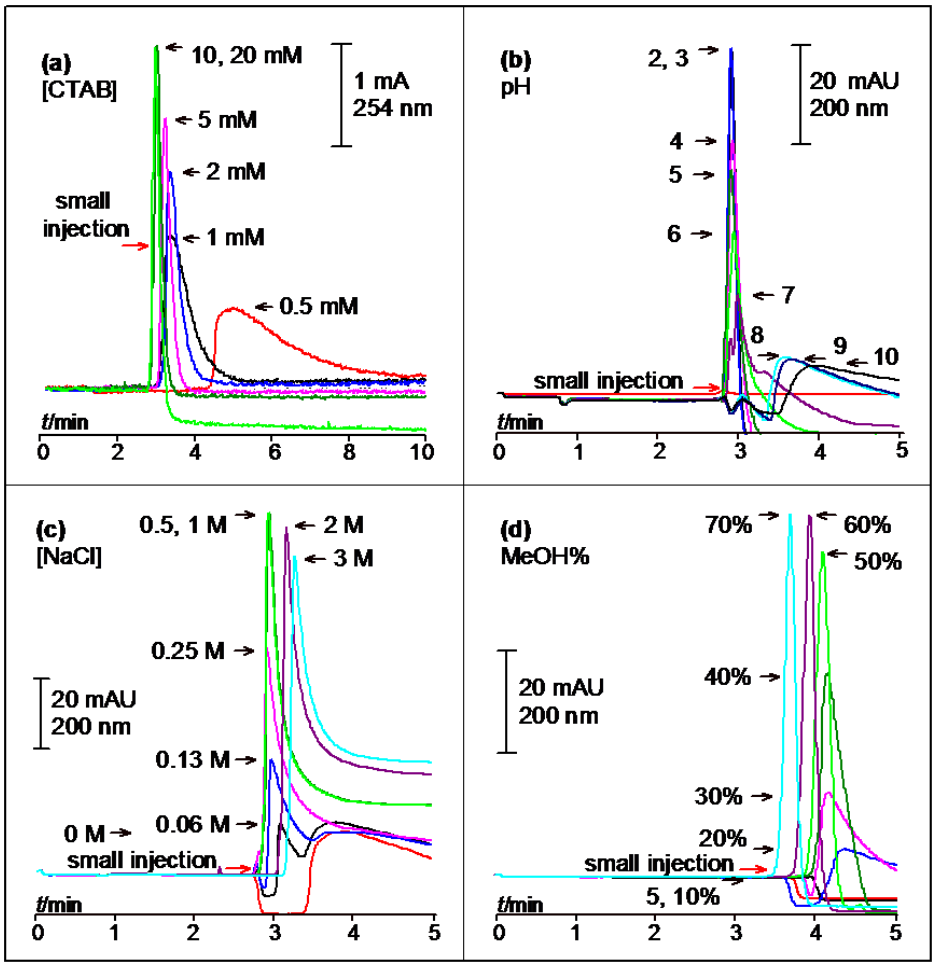

Figure S2. Sample enrichment of a tripeptide by varying the [CTAB] from 0.5-20 mM (a), pH from 2-10 (b), [NaCl] from 0-3 M (c) and MeOH% from 5-70% (d) in the mobile phase. For sample enrichment (black arrows), 0.06 mM tyr-tyr-tyr in 0.5 mM CTAB and 100 mM ammonium bicarbonate pH 8.5 buffer (sample matrix) was loaded at a length of 33.4 cm. The 50 µm i.d. capillary (60 cm total length) was conditioned with the sample matrix. For small injection (red arrows), 0.06 (b-d) or 2 (a) mM tyr-tyr-tyr in buffer was injected at a length of 2 mm. The capillary was conditioned with buffer. Mobilisation with the mobile phase (sample enrichment) or buffer (small injection) was at a flow rate of 16.7 cm/min. 100 mM ammonium bicarbonate pH 8.5 was also the buffer in (a), (c), and (d) and phosphate solutions at different pH values were buffers in (b). Enrichment with NaCl (*i.e.*, $\geq$ 0.5 M NaCl in 100 mM ammonium bicarbonate pH 8.5 buffer), although still effective, was the weakest and gave a broad concentrated peak compared to the sharp peaks obtained with the other mobile phases. The increase in the amount of solution micelles due to the lowering of the cmc by the addition of salt was not as straightforward as when the surfactant was added into the mobile phase. The β_{mc} of 0.5 mM CTAB in 0.5 M NaCl and buffer was considered comparable to that of 2 mM CTAB in buffer, based on the observed peak widths.

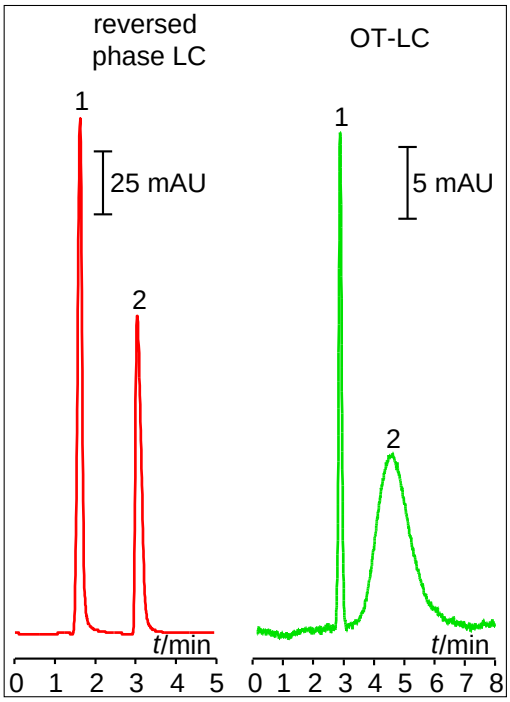

Figure S3. Gradient reversed phase LC and OT-LC of paracetamol (peak 1) and mefenamic acid (peak 2) drug product. Detection was at 260 nm. A 20 μL aliquot of 30 μg/mL of each drug in initial mobile phase composition was injected in reversed phase LC. LC mobile phase (initial and gradient) conditions are stated in the text. A 2 mm plug of sample (100 μg/mL each drug) prepared in mobile phase was pressure injected in OT-LC. Mobile phase was 1.5 mM CTAB in 50 mM sodium phosphate pH 7.5 in OT-LC. The capillary was 50 μm i.d. and 60 cm total length. More explanation in the text.