pharmaceuticals

SUPPLEMENTARY MATERIALS

Table S1. Chromatographic parameters for paracetamol (50 µg/L), caffeine (50 µg/L) and tramadol (50 µg/L) based on the proposed method (mobile phase composition: 40% MeOH; pH = 4.5; Phosphate concentration = 6 mM; Flow rate = 1.0 mL/min; dead time, t_o = 2.431 min). (*) P: paracetamol; C: caffeine; T: tramadol.

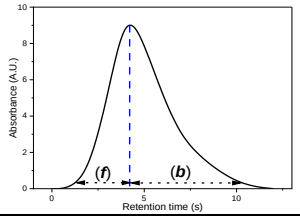
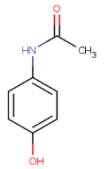
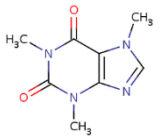
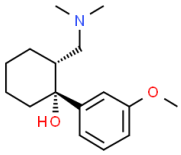
Parameter	Equation	Analyte			
		Paracetamol (40:60)	Caffeine (40:60)	Tramadol (40:60)	Reference value
b		0.179	0.199	0.256	
f		0.080	0.100	0.133	
TF	$= (b+f)/2f$	1.619	1.495	1.462	
AF	$= (b/f)$	2.24	1.99	1.92	$T \leq 2$
t_R		3.873	5.228	6.685	
t'_R	$= t_R - t_o$	1.442	2.797	4.254	
k'	$= t'_R/t_o$	0.593	1.151	1.750	$1 < k' < 10$
$\alpha_{2/1}$	$= \frac{(t'_R)_2}{(t'_R)_1} = \frac{k'_2}{k'_1}$		1.940 (P-C)*	1.521 (C-T)* 2.950 (P-T)*	$\alpha > 1$
N_a* (asim)	$= 41{,}7\ \frac{\left(\frac{t'_R}{W_{0.1}}\right)^2}{\left(\frac{f_{0.1}}{b_{0.1}} + 1.25\right)}$	762	2082	2818	N > 2000
H* (asim)	$= L/N_a$*	0.03282	0.01201	0.00887	
R_a' (asim)	$= \sqrt{\frac{\bar{N}}{16}}\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{\bar{k}}{1 + \bar{k}}\right]$		2.002 (P-C)*	2.494 (C-T)* 3.413 (P-T)*	$R > 2$

Table S2. A comparison of the limits of detection (LOD) and quantification (LOQ) achieved by the developed method against other publications.

Paracetamol 		**Caffeine** 		**Tramadol** 		**Detector (λ, nm)**	**Reference**
LOD (µg/mL)	**LOQ (µg/mL)**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**LOD (µg/mL)**	**LOQ (µg/mL)**		
0.2	0.8	0.1	0.4	0.3 (0.1)*	1.0 (0.2)*	UV-Vis (210)	This work
0.03	0.1	-	-	-	-	Fl* (355)	[11]
0.004	-	-	-	-	-	SWV	[12]
1.0	10.0	-	-	-	-	UV-Vis (210)	[13]
-	-	0.100	0.250	-	-	UV-Vis (215)	[14]
-	-	0.152	0.461	-	-	UV-Vis (272)	[16]
-	-	-	-	0.7	0.9	UV-Vis (218)	[17]
0.467	1.415	0.752	2.277	-	-	UV-Vis (λ ?)	[19]
0.409	1.226	0.151	0.502	-	-	UV-Vis (215)	[20]
0.09	0.29	0.17	0.56	-	-	UV-Vis (207)	[21]
0.67	-	0.13	-	-	-	UV-Vis (220)	[22]
0.18	0.53	0.220	0.601	-	-	UV-Vis (210)	[24]
0.64	2.13	-	-	1.36	4.53	UV-Vis (220)	[25]
20.00	66.70	-	-	6.00	20.00	GC-MS	[25]
0.008	0.016		-	1.317	2.634	DPV	[26]
2.589	7.847	-	-	0.479	1.453	UV-Vis (281)	[28]
0.035	0.116	0.019	0.062	-	-	SWV	[29]
35.8	108.5	-	-	4.14	12.54	UV-Vis (271)	[30]
0.57	1.74	-	-	0.31	0.95	UV-Vis (270)	[31]
0.012	-	0.019	-	0.132	-	DPV	[38]

*Using fluorescence detection system; SWV: Square Wave Voltammetry; DPV: Differential Pulse Voltammetry

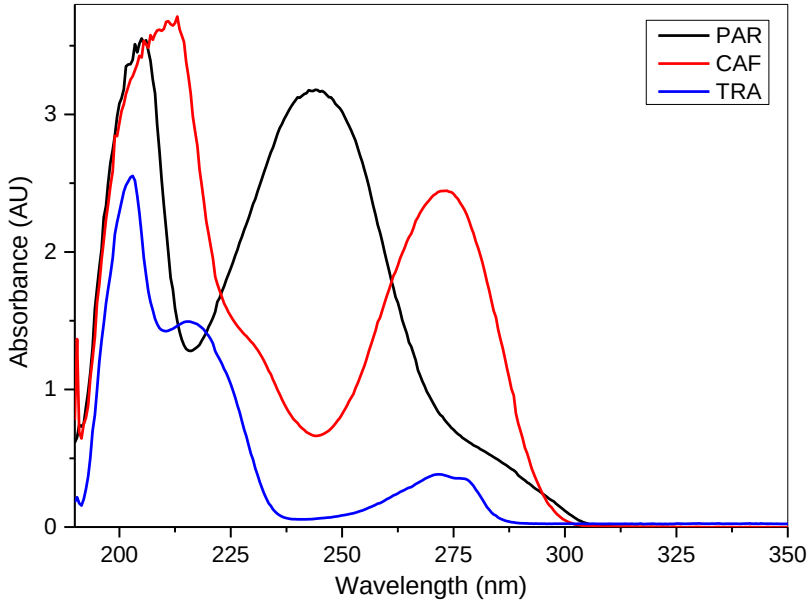


Fig. S1. UV-Vis spectra of the three analytes at the 10 μg/mL concentration in aqueous solution (AU: arbitrary units).

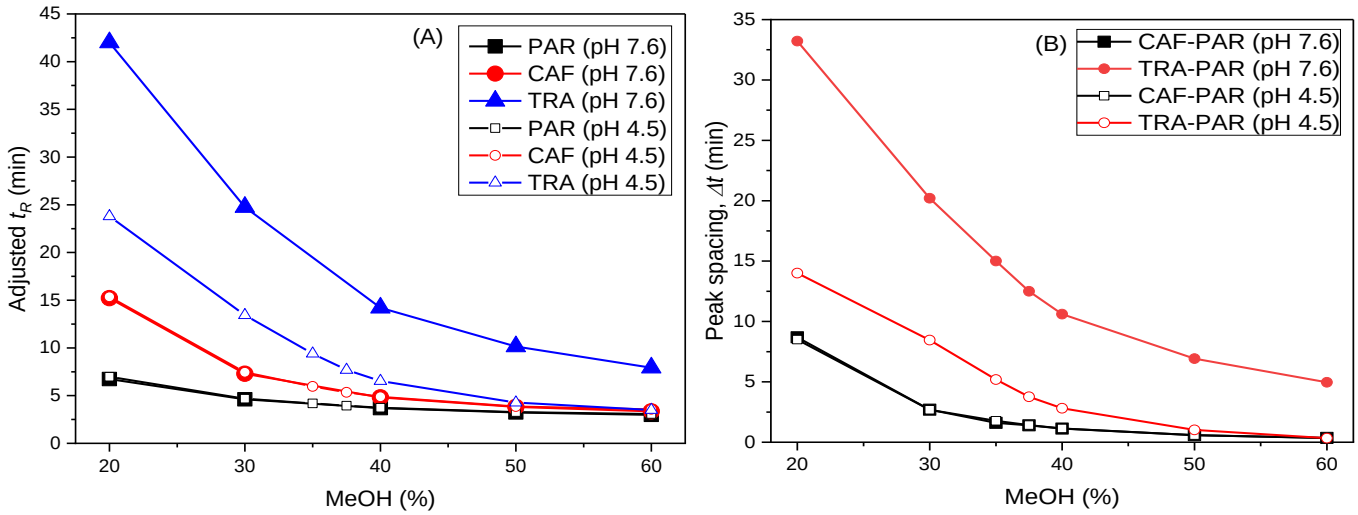


Fig. S2. (A) Adjusted retention time and (B) Peak spacing against the composition of the mobile phase at two pH values.

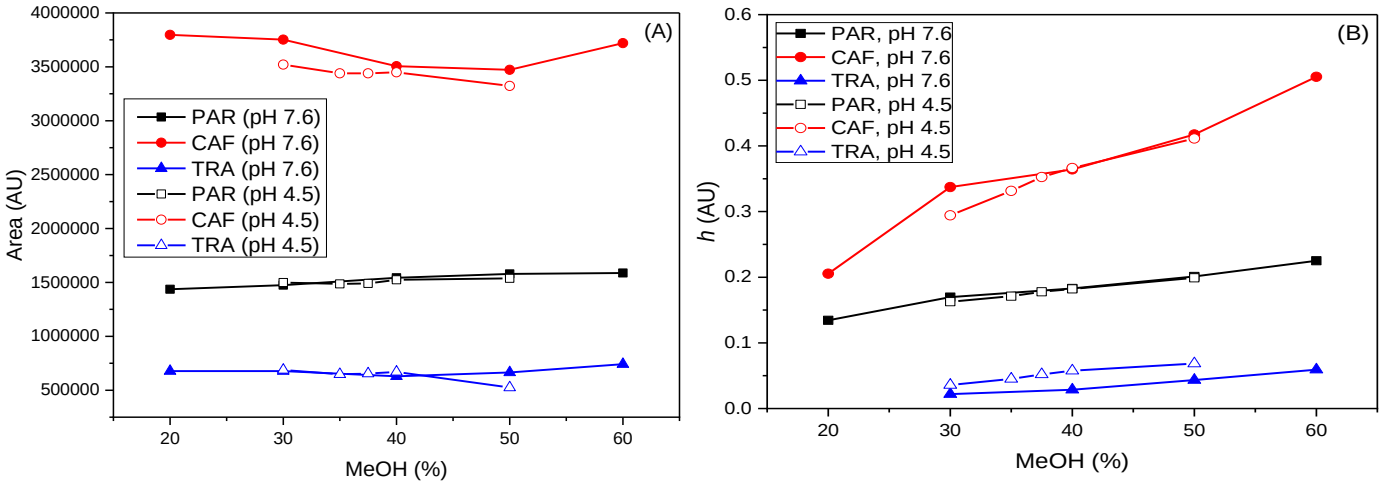


Fig. S3. (A) Peak area and (B) peak height against the composition of the mobile phase at two pH values (AU: arbitrary units).

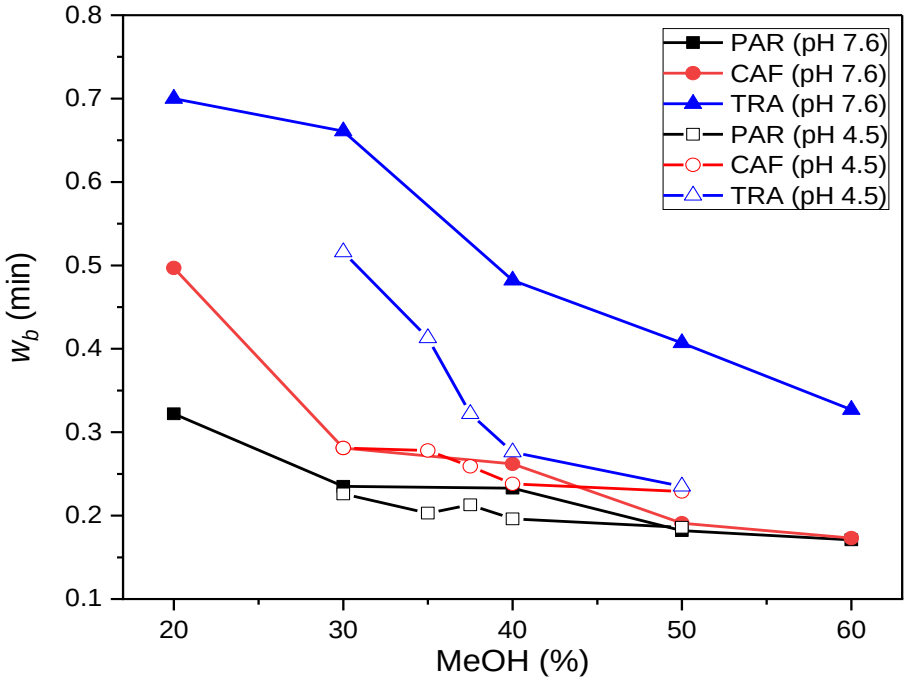
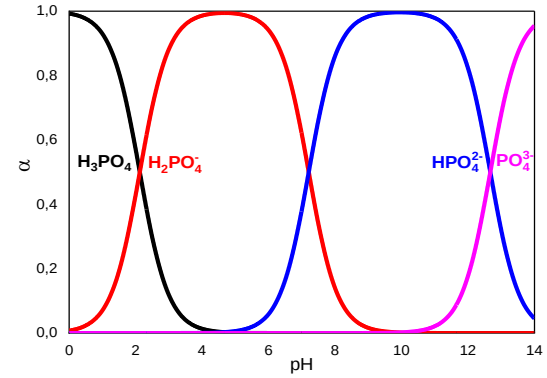
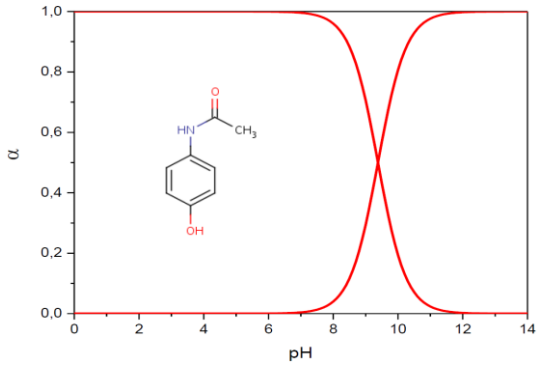


Fig. S4. Peak base-width against the composition of the mobile phase at two pH values.

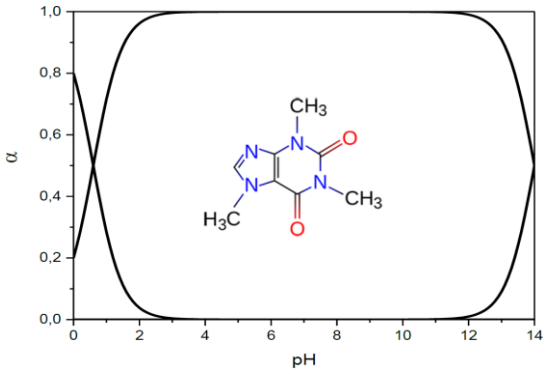
Phosphoric acid



Paracetamol



Caffeine



Tramadol

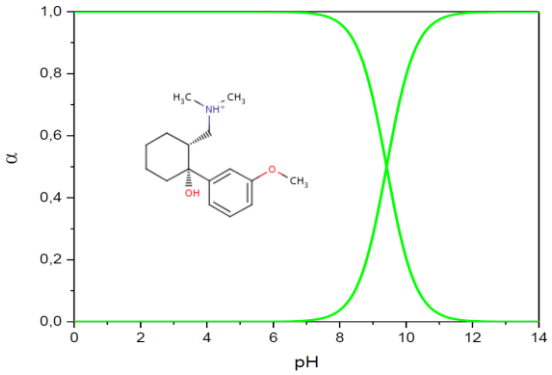


Fig. S5. Distribution diagram of phosphoric acid, paracetamol, caffeine and tramadol species as a function of pH.

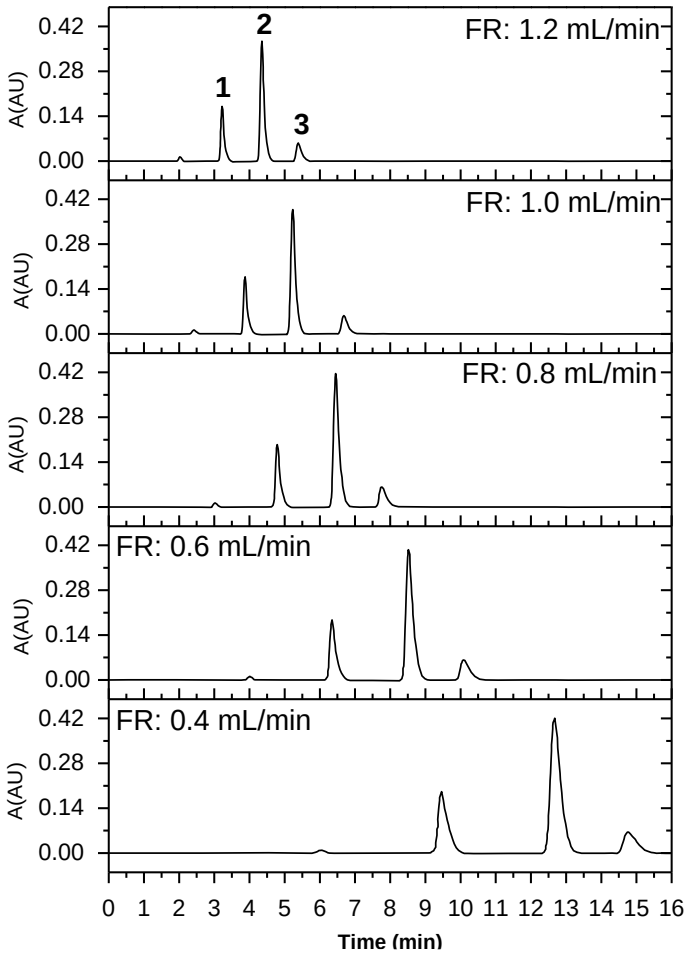


Fig. S6. Chromatograms obtained for several flow rates (FR) of the mobile phase using the selected 40% MeOH and pH 4.5 (1: paracetamol; 2: caffeine; 3: tramadol). Other parameters as in Fig. 1 (AU: arbitrary units).

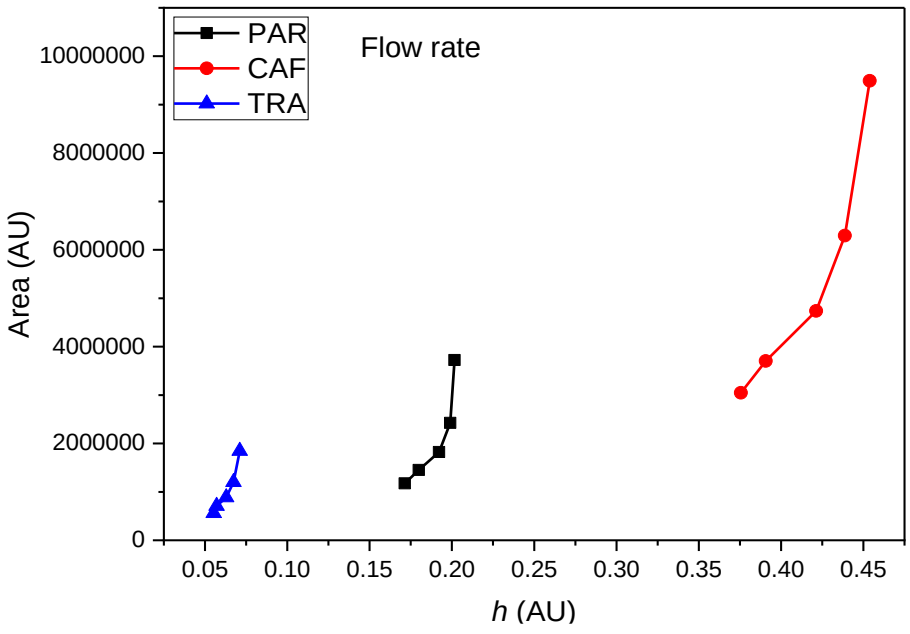


Fig. S7. Peak area against peak height for the three analytes and five flow rate values (AU: arbitrary units).

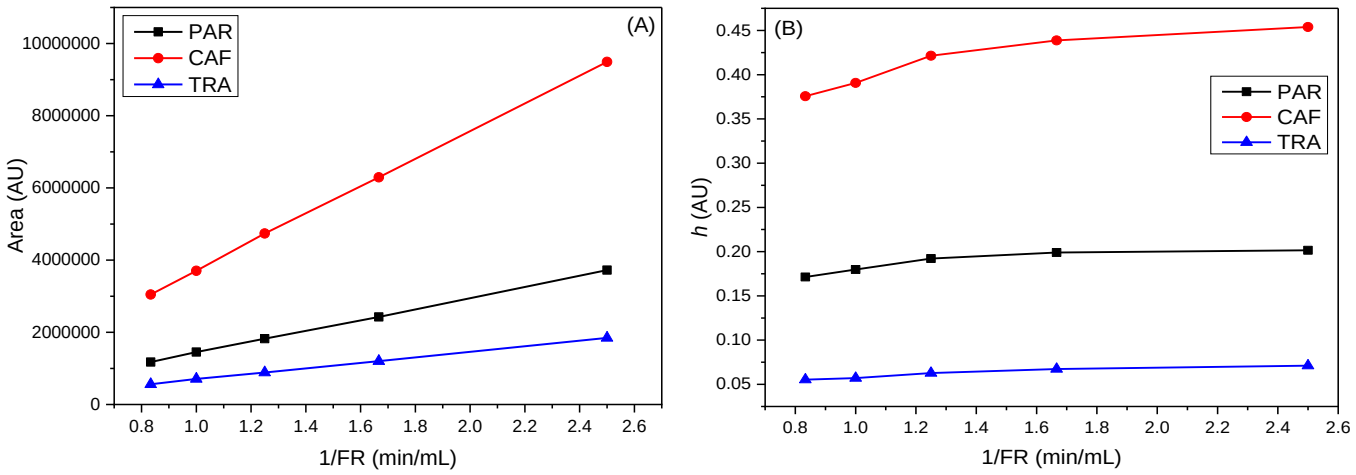


Fig. S8. (A) Peak area and (B) peak height against the inverse of the flow rate for the three drugs (AU: arbitrary units).

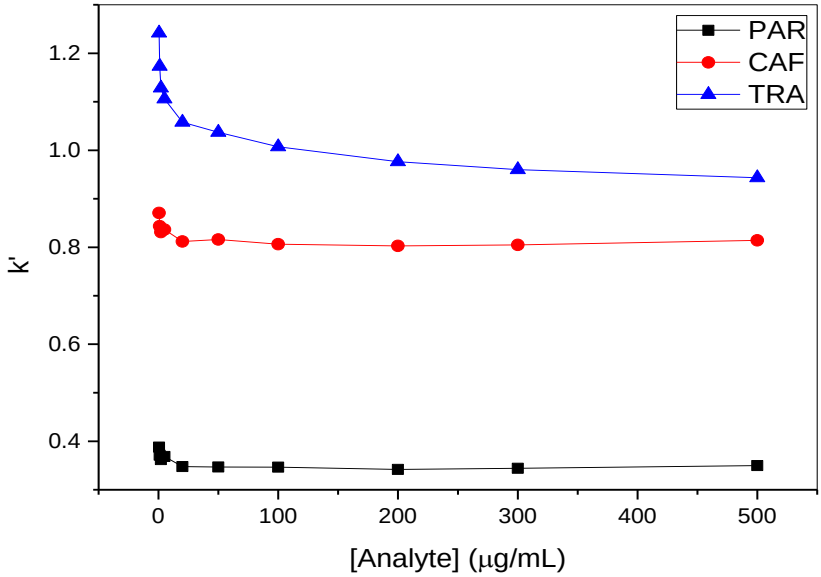


Fig. S9. Capacity factor against the analyte concentration for the three drugs.

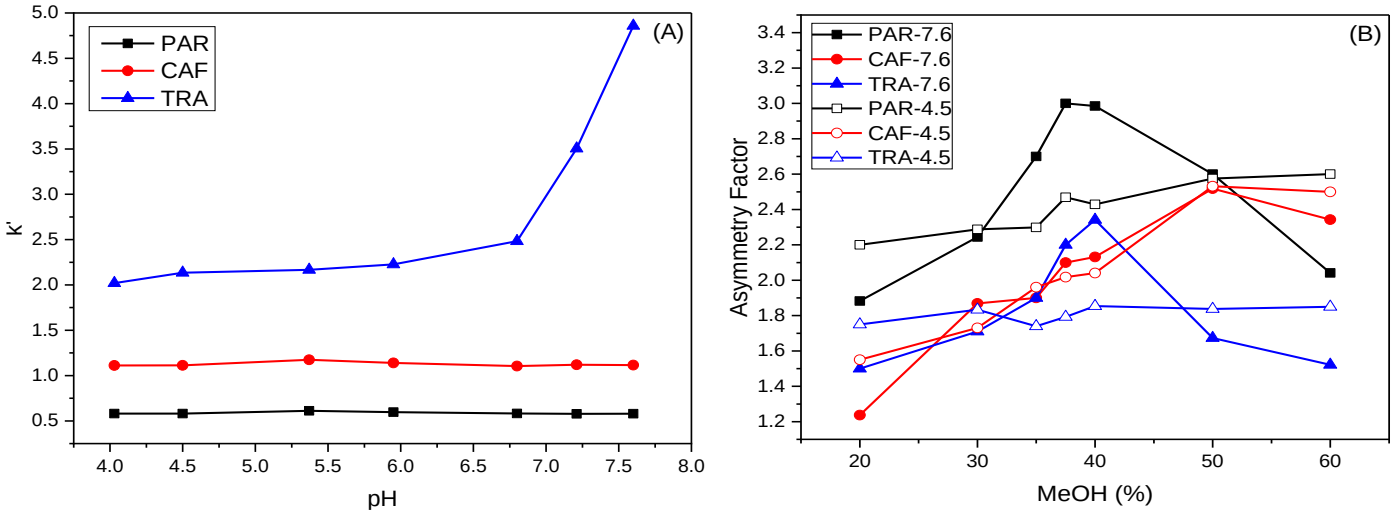
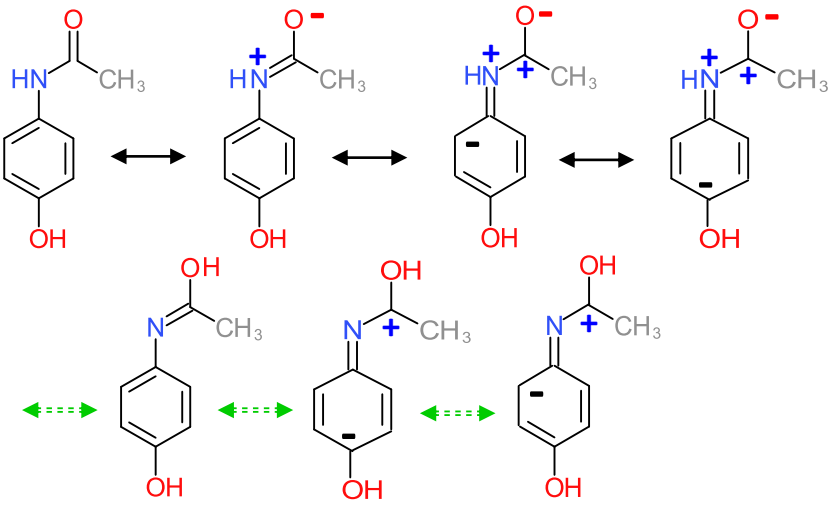
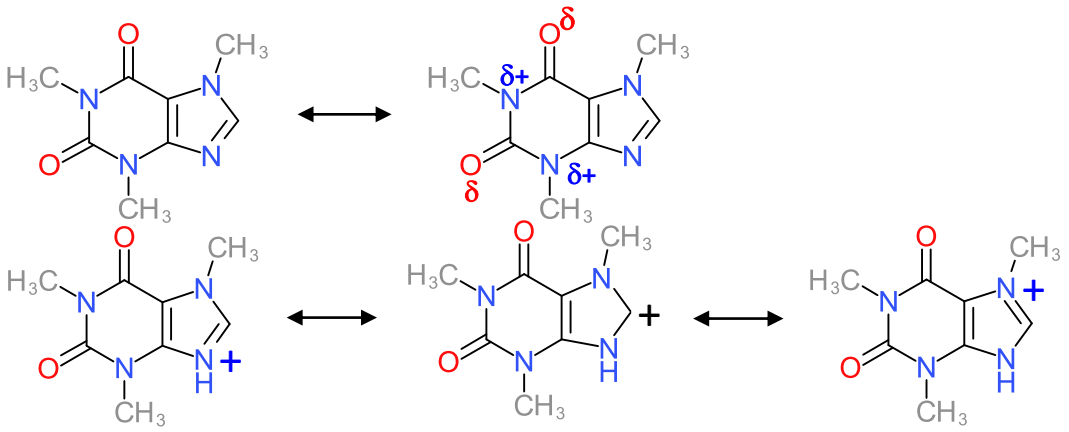


Fig. S10. Plots of (A) the capacity factor *vs.* pH and (B) the asymmetry factor *vs.* composition of the mobile phase at two pH values.

Paracetamol



Caffeine



Tramadol

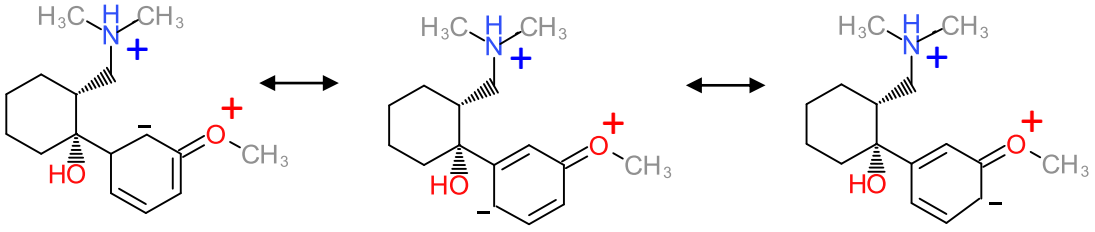
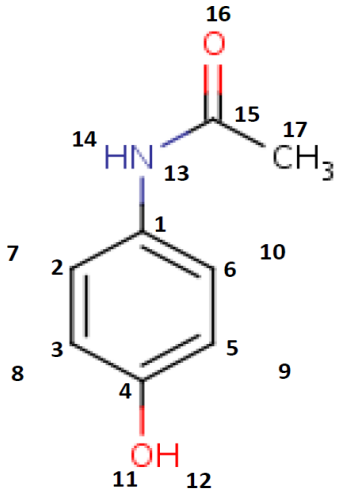
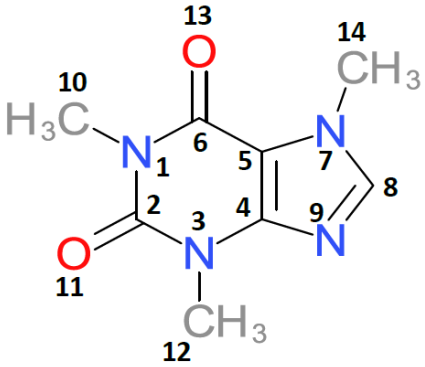


Fig. S11. Resonant structures for paracetamol (for two tautomers), caffeine (neutral and protonated forms) and tramadol (protonated form).

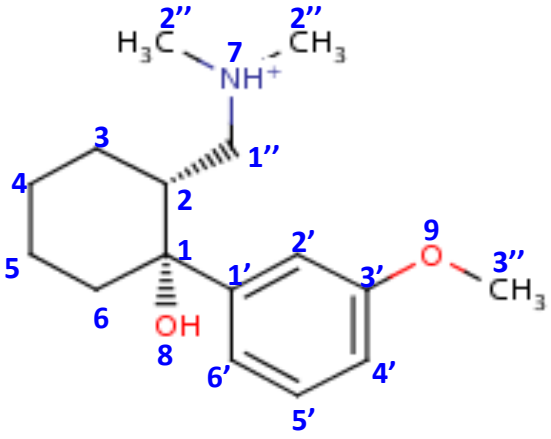
Paracetamol



Caffeine



Tramadol hydrochloride



Cl-

Fig. S12. Numeration of the carbon atoms.