Electronic Supplementary Material (ESI) for Analyst.

Synthesis and characterization of a multimode stationary phase: Congo red derivatized silica in nano-flow HPLC

The retention factors (k'), separation factors (α), resolution values (R_S) and theoretical plate number (N) were calculated from Equation (1), Equation (2), Equation (3) and Equation (4), respectively.

$$k' = \frac{t_{R(A)} - t_M}{t_M} \quad (1)$$

$$\alpha = \frac{t_{R(B)} - t_M}{t_{R(A)} - t_M} \quad (2)$$

$$R_S = \frac{t_{R(B)} - t_{R(A)}}{W_{1/2(B)} + W_{1/2(A)}} \quad (3)$$

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 = 16\left(\frac{t_R}{W}\right)^2 \quad (4)$$

where t_M is the column void time which was determined by uracil, $t_{R(A)}$ is the retention time for the faster moving analytes and $t_{R(B)}$ for the slower, meanwhile $W_{1/2(A)}$ and $W_{1/2(B)}$ is the corresponding peak width at half height, W is the corresponding peak width.

Table S1 Retention factor (k'), resolution (Rs), asymmetry factor (As), and column efficiency (N) of each analyte for the separation of mixture on the Sil-CR packed capillary nano-flow HPLC column. Column 50 cm ×100 μm, 22 cm effective length, pump flow: 0.03mL/min (Experimental parameters see Figure 3).

Groups	Analytes	k'	Rs	As	N (plates m-1)
1	uracil	-	-	1.47	97395
2	4-hydroxybenzoic acid	0.134	1.42	1.23	80877
3	benzoic acid	0.188	1.52	1.32	81181
4	benzene	0.458	6.91	0.97	90440
5	toluene	0.572	2.58	1.03	85877
6	ethylbenzene	0.703	2.59	1.05	72563

Table S2 Reproducibility column efficiency and retention factor on column Sil-CR

	Column efficiency(%RSD)						Retention factors(%RSD)					
	uracil	4-hydroxybenzoic acid	benzoic acid	benzene	toluene	ethylbenzene	uracil	4-hydroxybenzoic acid	benzoic acid	benzene	toluene	ethylbenzene
Run-to-Run(n=6)	1.7	2.4	2.8	1.8	2.2	2.6	1.1	1.2	1.7	1.4	1.8	2.1
Day-to-Day(n=3)	2.1	2.6	3.1	2.2	2.5	2.9	1.3	1.6	2.2	2.0	2.4	2.7

Experimental conditions: columns, 25.0 cm effective length, 33 cm total length with 100 □m id and 375 □m od; mobile phase, 10 mM phosphate buffer, pH 8.0,

V

ACN

/V

buffer

= 50:50; injection, electrokinetic injection with 10 kV and 10 s; separation voltage, 15 kV; detection wavelength 214 nm.

Experimental conditions: columns, 25.0 cm effective length, 33 cm total length with 100 □m id and 375 □m od; mobile phase, 10 mM phosphate buffer, pH 8.0,

V

ACN

/V

buffer

= 50:50; injection, electrokinetic injection with 10 kV and 10 s; separation voltage, 15 kV; detection wavelength 214 nm.

Experimental conditions: columns, 22 cm effective length, 50 cm total length with 100 μm id and 375 μm od; mobile phase, 12.5mM sodium acetate (pH = 4.2) buffer/acetonitrile (80:20, v/v)

Table S3 The degree of deprotonation (D.D.%) and the pKa of benzoic acid compands (Experimental parameters see Figure 5)

Peak	Analytes	$pK_a^{\#a}$	D.D. (%)
1	4-hydroxybenzoic acid	4.57±0.10	80.28
2	protocatechuic acid	4.45±0.10	84.30
3	m-toluic acid	4.27±0.10	89.05
4	benzoic acid	4.20±0.10	90.52
5	2, 4-dihydroxybenzoic acid	3.32±0.10	98.64
6	2-hydroxybenzoic acid	2.98±0.10	99.38

#a Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs) and provided by SciFinder.

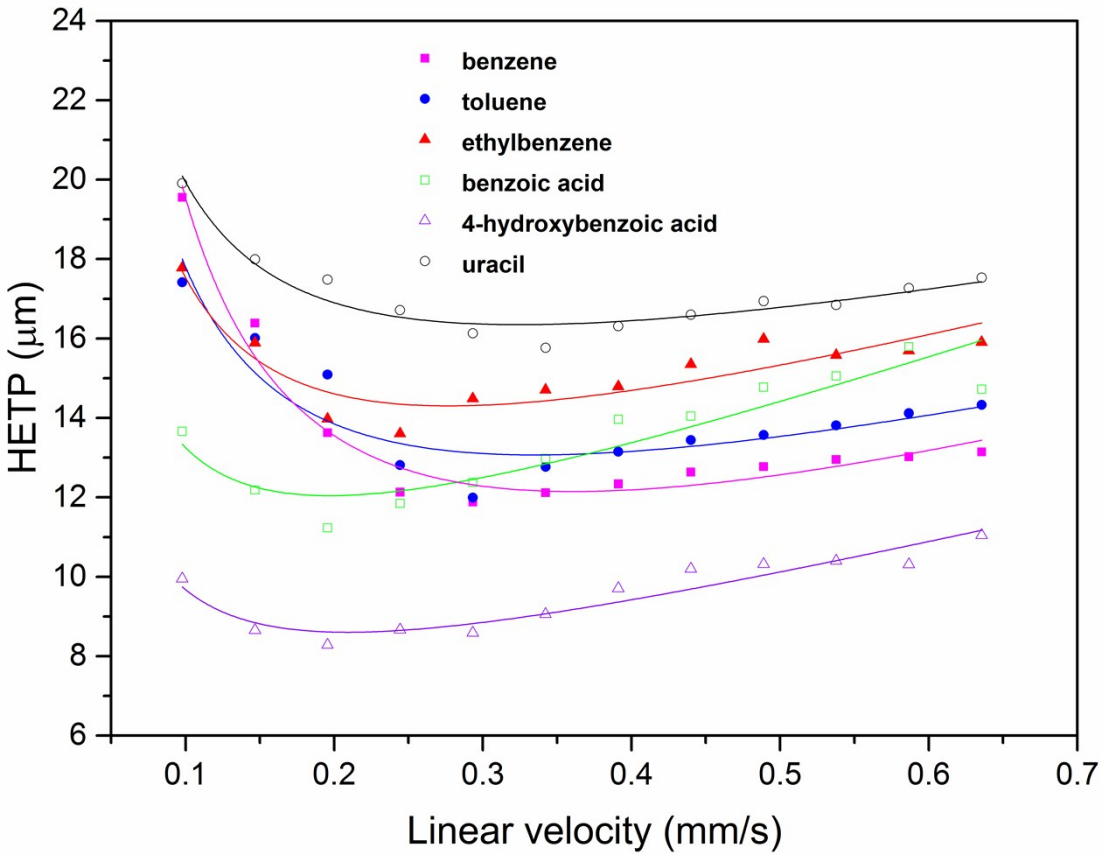

Fig. S1 Linear velocity and plate height curves of uracil, 4-hydroxybenzoic acid, benzoic acid, benzene, (5) toluene and ethylbenzene on Sil-CR column with 12.5mM sodium acetate (pH = 4.2) buffer/acetonitrile (80:20, v/v). Column: 50 cm ×100 μm, 22 cm effective length, λ = 254 nm.

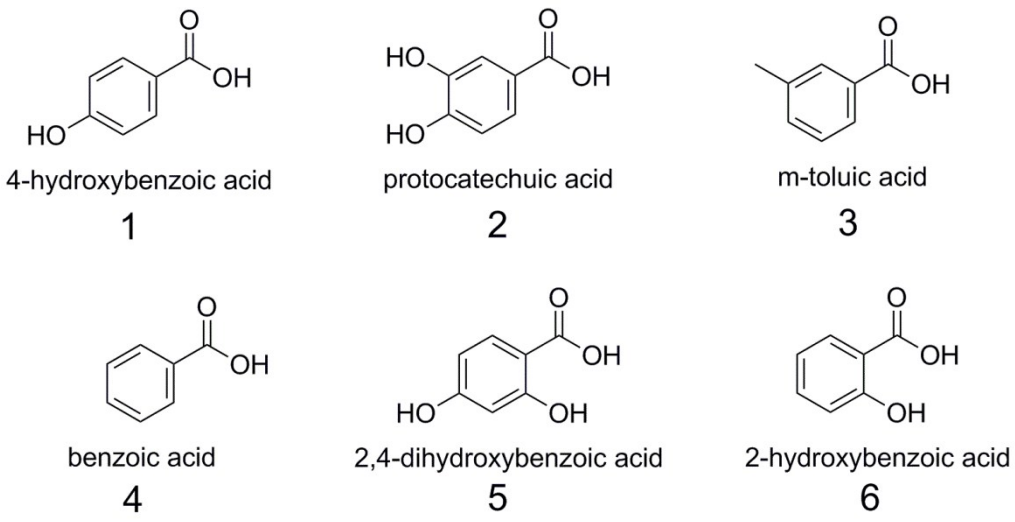

Fig. S2 The structures of six benzoic acid componds: 1) 4-hydroxybenzoic acid, 2) protocatechuic acid, 3) m-toluic acid, 4) benzoic acid, 5) 2, 4-dihydroxybenzoic acid and 6) 2-hydroxybenzoic acid

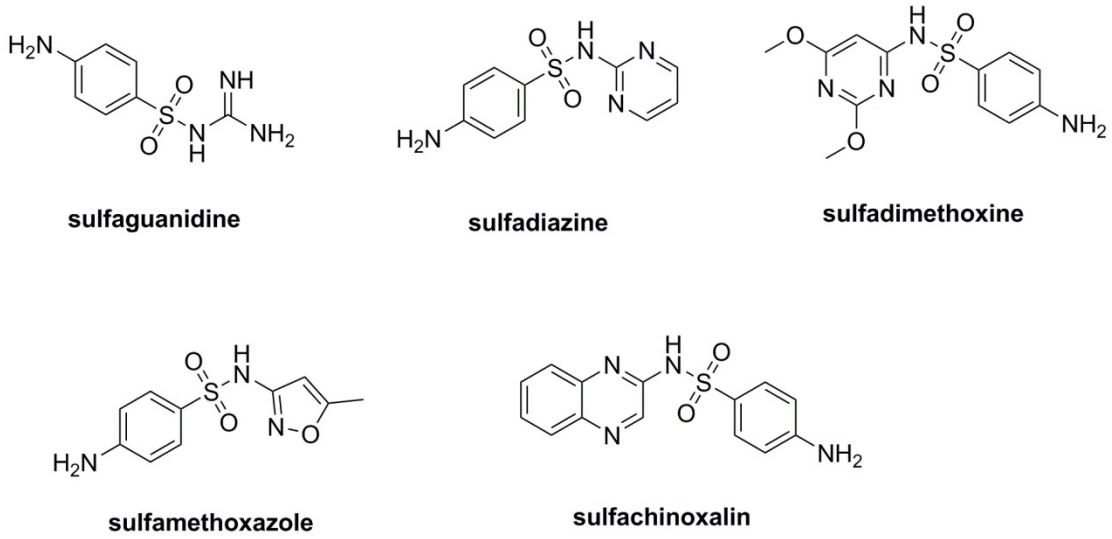

Fig. S3 The structures of five sulfonamides: 1) sulfaguanidine, 2) sulfadiazine, 3) sulfamethoxazole, 4) sulfadimethoxine, 5) sulfachinoxalin