Electronic Supplementary Information for

Preparation and application of a novel mixed-mode monolith for reversed-phase and per aqueous capillary electrochromatography

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Calculations

(1). The retention factor (k') was calculated using the following equation [1-4]:

$$k' = \frac{t_m\left(1 + \frac{\mu_{ep}}{\mu_{eo}^{*}\frac{i_{open}}{i_{momolith}}}\right) - t_0}{t_0}$$

where t_0 and t_m are the retention time of the charged analyte and the EOF marker, respectively; μ_{ep} is the electrophoretic mobility of the charged analyte; $\mu_{eo}$is the actual "interstitial" electroosmotic mobility of the eluent in the monolithic column. The value of μ_{eo} is obtained by multiplying the "apparent" electroosmotic mobility μ_{eo}^{*} of the monolith by the tortuosity factor of the column. This tortuosity factor is determined from the ratio of the currents observed in the CZE (i_{open}) and CEC ($i_{momolith}$) modes for the same running conditions [3].

For a neutral analyte, μ_{eo}= 0, and thus the k' of the neutral analytes could be expressed by the following equation:

$$k' = \frac{t_m - t_0}{t_0}$$

(2). The linear flow velocity of EOF (v_{EOF}) was calculated using the following equation:

$$v_{EOF} = \frac{L_e}{t_0}$$

where L_e is the effective length of the monolithic column; t_0 is the retention time of the EOF marker.

(3). The electroosmotic mobility (μ_{EOF}) was calculated using the following equation [5]:

$$\mu_{EOF} = \frac{\nu_{EOF}}{E}$$

where *E* is the electric field strength.

According to the equation (2), μ_{EOF} could be expressed by the following equation [6]:

$$\mu_{EOF} = \frac{\nu_{EOF}}{E} = \frac{L_e/t_0}{E} = \frac{L_e L_t}{t_0 V}$$

where L_e and L_t are the effective length and the total length of the monolithic column, respectively; t_0 is the retention time of the EOF marker; *V* is the applied voltage.

(4). The plate height (*H*) was calculated using the following equation:

$$H = \frac{L_e}{N}$$

where L_e is the effective length of the monolithic column, *N* is is the theoretical plate number.

References

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2. A. S. Rathore, E. Wen and C. Horvath, *Anal. Chem.*, 1999, **71**, 2633-2641.

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4. J. He, X. Wang, M. Morill and S. A. Shamsi, *Anal. Chem.*, 2012, **84**, 5236-5242.

5. A. Van De Goor, B. Wanders and F. Everaerts, *J. Chromatogr. A*, 1989, **470**, 95-104.

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Characterization

The quantitative evaluation of 4,5-imidazoledicarboxylic acid on the surface of the IDS monolithic matrix was also made by using the following equation: the coverage of imidazolium groups (μmol m^{-2}) = (N%×10^4)/(28×S), where N% represents the percentage of nitrogen as determined by elemental analysis (1.58%), S is the specific surface area of the IDS hybrid monolith (634.84 m^2 g^{-1}). The average content of the bonded 4,5-imidazoledicarboxylic acid on the surface of the monolith was calculated to be 0.89 μmol m^{-2}.

Figures

Fig. S-1. Effect of ACN content on the retention of nucleosides and nucleotide bases on the IDS hybrid monolithic column. Conditions: 20 mM NaH_2PO_4 buffer at pH 3.0 with different ACN contents; applied voltage, -15 kV; detection wavelength, 214 nm.

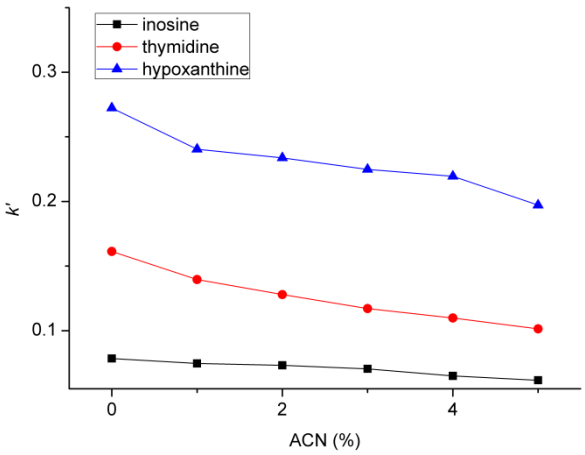


Fig. S-2. Effect of mobile phase pH on the retention of nucleosides and nucleotide bases on the IDS hybrid monolithic column. Conditions: 10 mM NaH_2PO_4 buffer at different pH; applied voltage, -15 kV; detection wavelength, 214 nm.

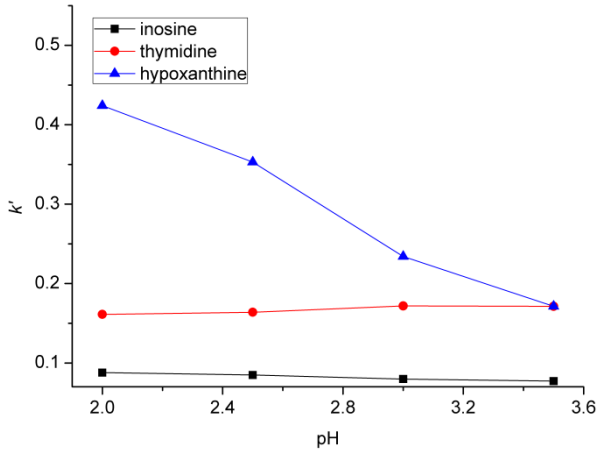


Fig. S-3. Effect of ACN content on the retention of amino acids on the IDS hybrid monolithic column. Conditions: 10 mM NaH_2PO_4 buffer at pH 3.0 with different ACN contents; applied voltage, -20 kV; detection wavelength, 214 nm.

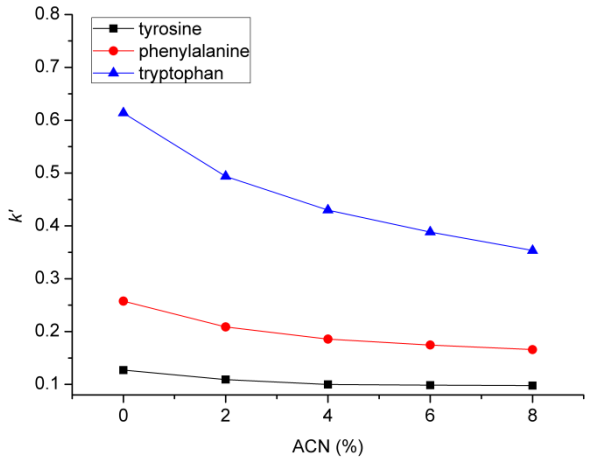


Fig. S-4. Effect of buffer concentration on the retention of amino acids on the IDS hybrid monolithic column. Conditions: various buffer concentrations at pH 3.0; applied voltage, -20 kV; detection wavelength, 214 nm.

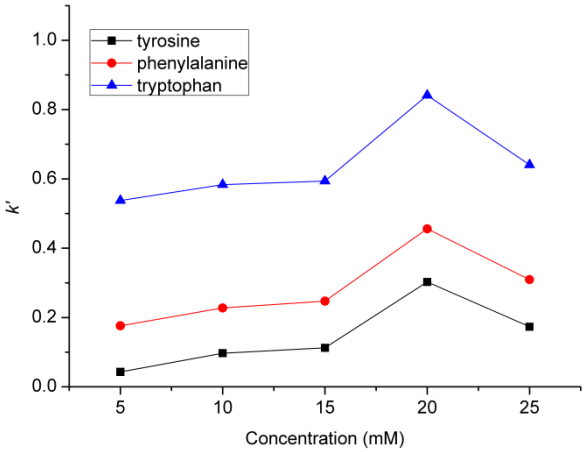


Fig. S-5. Effect of mobile phase pH on the retention of amino acids on the IDS hybrid monolithic column. Conditions: 10 mM NaH_2PO_4 buffer at different pH; applied voltage, -20 kV; detection wavelength, 214 nm.

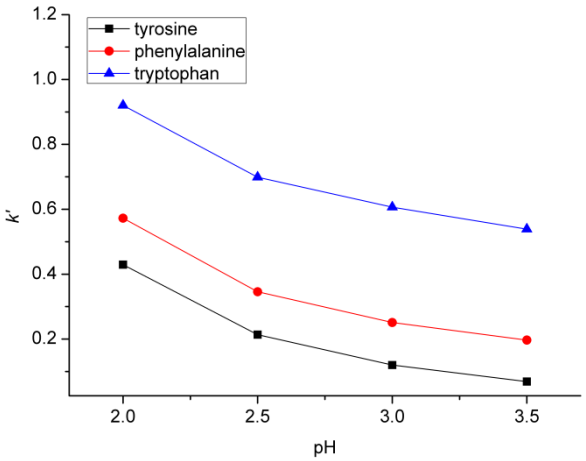


Fig. S-6. Effect of ACN content on the retention of phenols on the IDS hybrid monolithic column. Conditions: 10 mM NaH_2PO_4 buffer at pH 3.0 with different ACN contents; applied voltage, -20 kV; detection wavelength, 214 nm.

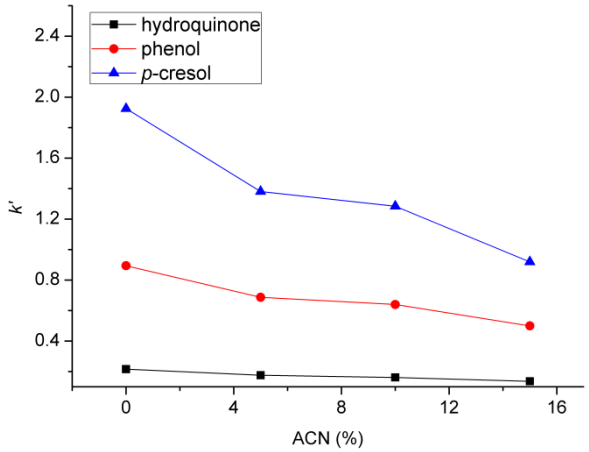
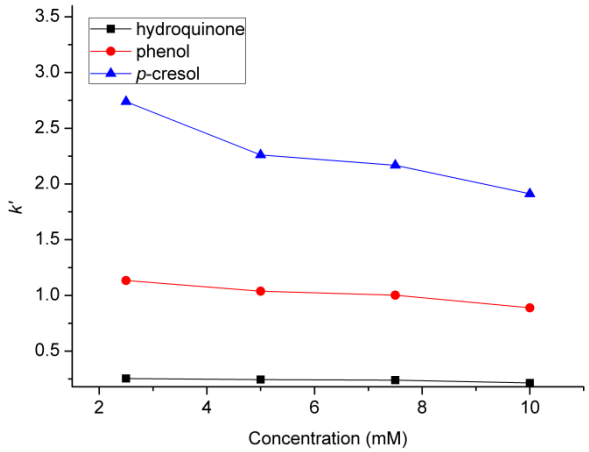


Fig. S-7. Effect of buffer concentration on the retention of phenols on the IDS hybrid monolithic column. Conditions: various buffer concentrations at pH 3.0; applied voltage, -20 kV; detection wavelength, 214 nm.



Tables

Table S-1. Retention factors (k') and column efficiencies (N m^{-1}) for nucleoside and nucleotide bases at various phosphate concentrations under the PACEC mode.

Solute	5 mM		10 mM		15 mM	
	k'	N m^{-1}	k'	N m^{-1}	k'	N m^{-1}
inosine	0.073	98 500	0.081	124 600	0.089	128 800
thymidine	0.158	78 300	0.155	100 800	0.151	104 500
hypoxanthine	0.265	124 000	0.422	135 500	0.645	118 000

Table S-2. Column efficiencies (N m^{-1}) for amino acids at different phosphate concentrations in the mobile phase under the PACEC mode.

Concentration (mM)	L-tyrosine	D,L-phenylalanine	L-tryptophan
5	45 500	34 800	16 300
15	69 800	45 800	21 500
25	80 300	55 000	31 800

Table S-3. Column efficiencies (N m^{-1}) for amino acids at different pHs under the PACEC mode.

pH	L-tyrosine	D,L-phenylalanine	L-tryptophan
2.5	82 500	52 500	25 300
3.0	79 300	49 300	24 000
3.5	72 500	47 000	23 500

Table S-4. Column efficiencies (N m^{-1}) for benzoic acid derivatives with different ACN contents in the mobile phase.

Solute	0%	5%	10%	15%	20%
***p*-hydroxybenzoic acid**	11 500	23 100	28 700	40 400	44 200
***p*-aminobenzoic acid**	13 100	27 400	31 000	46 000	49 500
benzoic acid	3 600	6 300	7 300	11 100	13 200

Table S-5. Column efficiencies (N m^{-1}) for benzoic acid derivatives at different pHs.

Solute	2.5	3.0	3.5	4.0	4.5
***p*-hydroxybenzoic acid**	31 300	29 000	25 300	14 800	8 000
***p*-aminobenzoic acid**	37 500	33 000	25 500	12 500	7 300
benzoic acid	10 000	9 300	7 500	3 800	1 800

Table S-6. Retention factors (k') and column efficiencies (N m^{-1}) for benzoic acid derivatives at different phosphate concentrations in the mobile phase.

Solute	5 mM		15 mM		25 mM	
	k'	N m^{-1}	k'	N m^{-1}	k'	N m^{-1}
***p*-hydroxybenzoic acid**	0.43	60 000	0.25	116 300	0.17	145 000

***p*-aminobenzoic acid**	0.47	40 500	0.45	126 000	0.34	130 500
benzoic acid	1.05	6 000	0.62	27 500	0.39	39 500