Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Evaluation of coverage, retention patterns, and selectivity of seven liquid chromatographic methods for metabolomics

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ESM I

1. Liquid chromatographic (LC) and mass spectrometric (MS) conditions
2. Retention factor distributions
3. Method orthogonality
4. Coverage of metabolite library subgroups

ESM II (separate file)

5. Metabolite library – full list of retention times and retention factors

216_2016_9716_MOESM2_ESM.xlsx

ESM I

1. Liquid chromatographic (LC) and mass spectrometric (MS) conditions

1.1 General MS Conditions:

Source: Dual Electrospray Ionization

Polarity	Positive (negative in method B)
Gas temperature	325 °C (C, D: 350 °C, E: 300 °C)
Drying gas	10 L/min (C, D: 12 L/min, E: 8 L/min)
Nebulizer	45 psig (C/D: 50 psig, E: 30 psig)
Sheath Gas Temp	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	4000 V (B, E: 3500 V)
MS-TOF Fragmentor	140 V
Skimmer	65 V
Mass range	50-1000 m/z
Acquisition rate	2 spectra/sec

1.2 LC gradients

Table S1 Columns and mobile phase conditions for methods A-G. * Note that methods C and D utilize the same column but different mobile phase modes

Column	A	B	C*	D*	E	F	G
Commercial Name	**Waters Acquity UPLC HSS T3**	**SeQuant ZIC pHILIC**	**Dionex Acclaim MM HILIC-1**	**Dionex Acclaim MM HILIC-1**	**SeQuant ZIC cHILIC**	**Waters XBridge Amide**	**Tosoh TSKgel NH2-100**
Type	**RP**	**Zwitterionic HILIC**	**Mixed-mode („neutral"), RP conditions**	**Mixed-mode („neutral"), HILIC conditions**	**Zwitterionic HILIC**	**"neutral" HILIC**	**„ion-exchange" HILIC**
Surface modification	**C18**	**sulfobetaine**	**alkyl diol**	**alkyl diol**	**phosphorylcholine**	**amide**	**alkylamine**
Dimensions/mm	2.1 x 100	2.1 x 150	4.6 x 150	4.6 x 150	2.1 x 150	3.0 x 150	2.0 x 150
Particle size/µm	1.8	5	5	5	3	3.5	5
Temperature / °C	55	45	30	30	40	40	40
Flow rate/µL*min^{-1}	450	300	700	700	300	700	200
Mobile phases A/B	H_2O/MeOH	H_2O/ACN	H_2O /ACN	H_2O /ACN	H_2O /ACN	H2O/ACN	H_2O /ACN
Ionic additive (channel)	0.1 % FA (A&B)	20 mM $(NH_4)_2CO_3$ (A)	0.1 % FA (A&B)	0.1% FA (A&B)	20 mM NH_4OAc (A&B)	0.1 % FA (A & B)	0.1 % FA (A)
Flow rate/µL*min-1	**450**	**300**	**700**	**700**	**300**	**700**	**200**
Gradient	min – % B 0 - 2 20 – 75 22 – 98 30 – 98 30.1 – 2 37.1 – 2	min – % B 0 – 95 2 – 95 15 – 60 17 – 60 17.1 – 5 27 – 5	min – % B 0– 5 30 – 75 31 – 5 40 – 5	min – %B 0 – 97 20 – 50 30 – 50 31 – 97 40 – 97	min – %B 0 – 80 15 - 20 20 – 20 20.1 – 50 30 – 50	min – %B 0 – 95 1.5 – 95 20 – 50 25 – 50 25.1– 95 35 – 95	min – %B 0– 90 2 – 90 17 – 50 22 – 50 22.1 – 90 32 – 90
Calculated t_0/min	0.62	1.39	2.84	2.84	1.39	1.2	1.9
MS mode	**ESI+**	**ESI-**	**ESI+**	**ESI+**	**ESI+**	**ESI+**	**ESI+**

2. Retention factor distributions

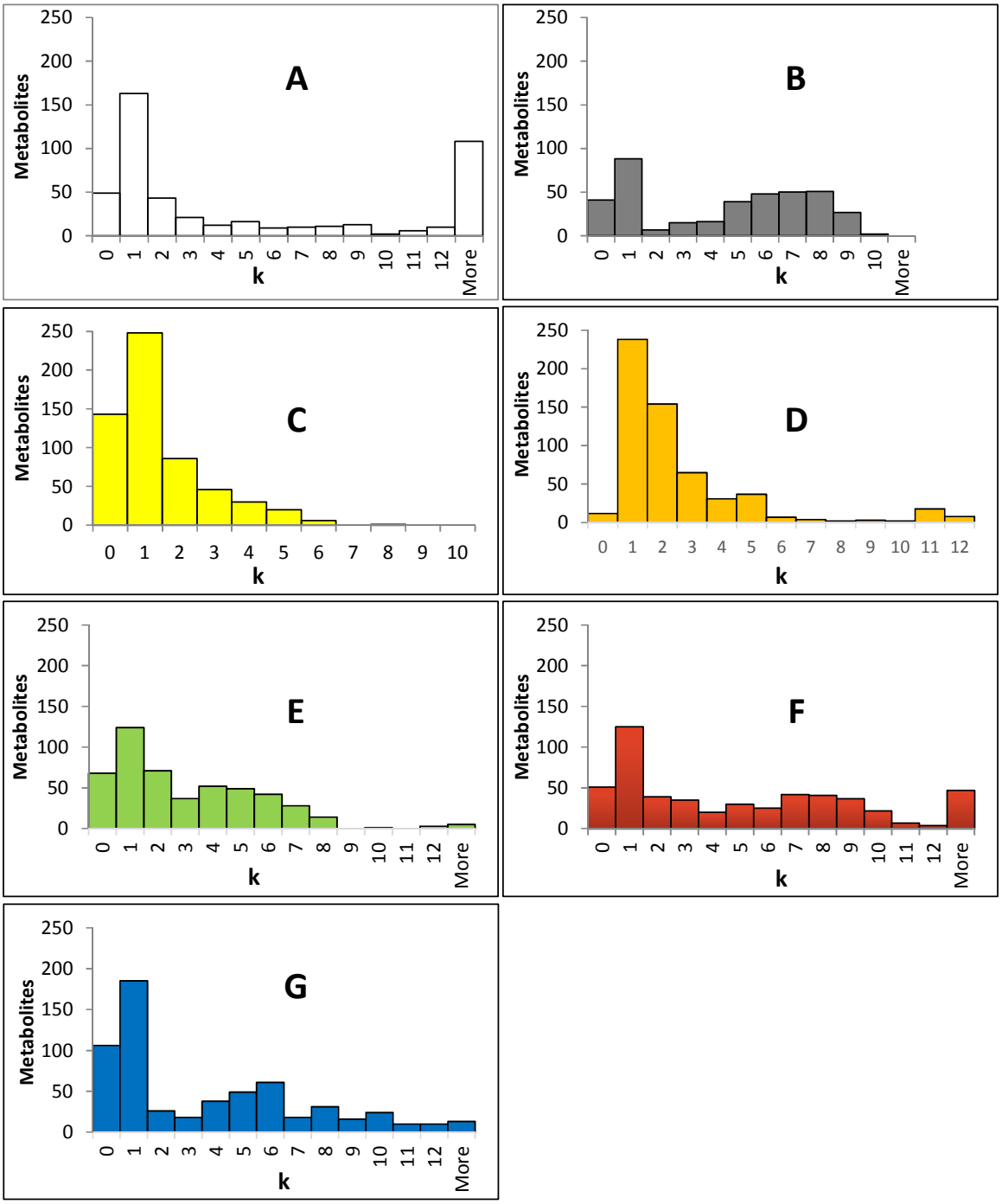

Fig. S1 Retention profiles for RP (A, C) and HILIC (B, D-G) methods based on retention factors of detected metabolite library compounds. Chromatographic conditions according to Table S1

3. Complementarity of HILIC methods

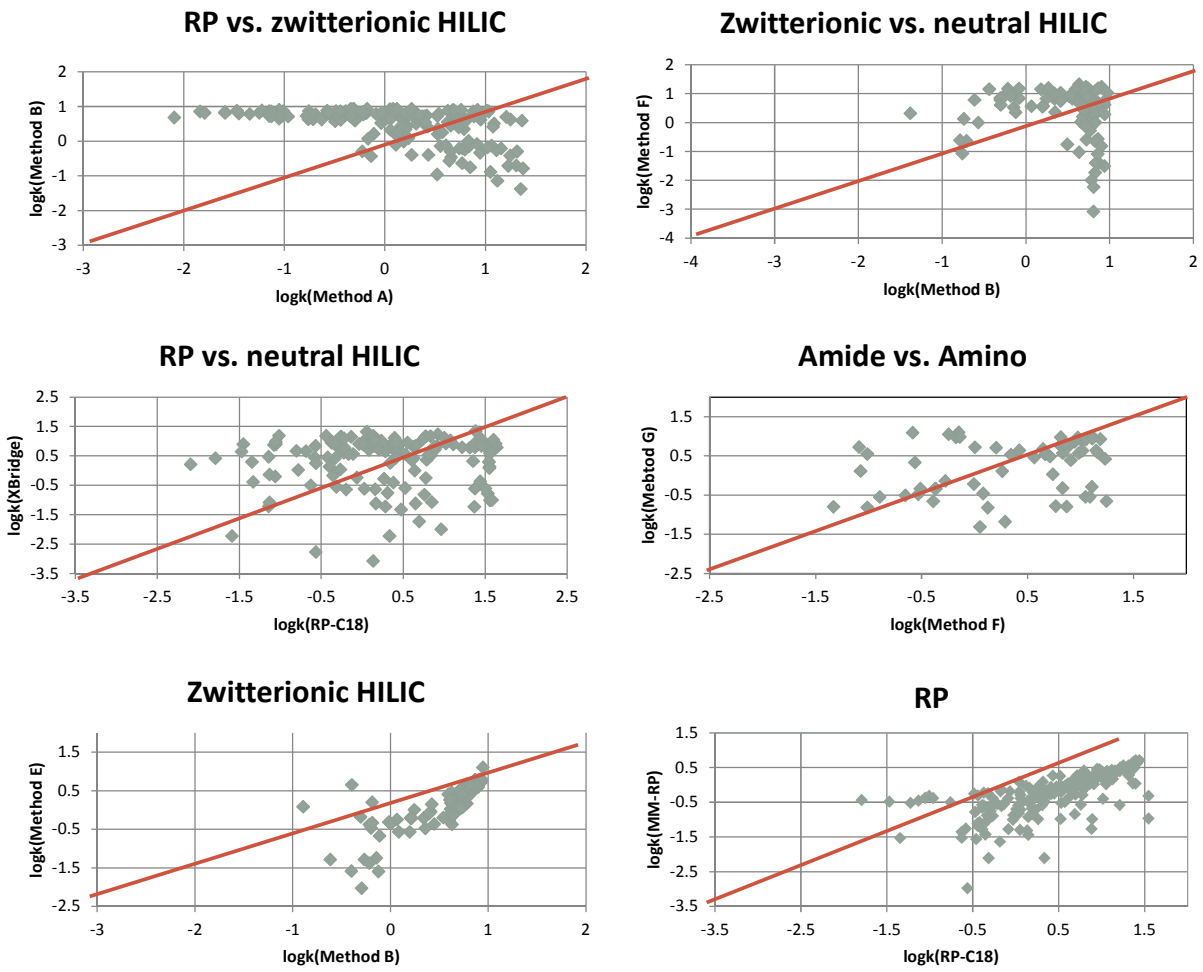

Fig. S2 Estimation of method orthogonality for RP and HILIC methods from plots of logarithmic retention factors. The deviation from the diagonal is larger for methods having high orthogonality (e.g., A/B or B/F, top row) compared to methods with similar selectivity (B/E, A/C, bottom row). Note that this comparison includes only compounds detected with both of the two methods being compared

4. Selectivity for metabolite library subgroups

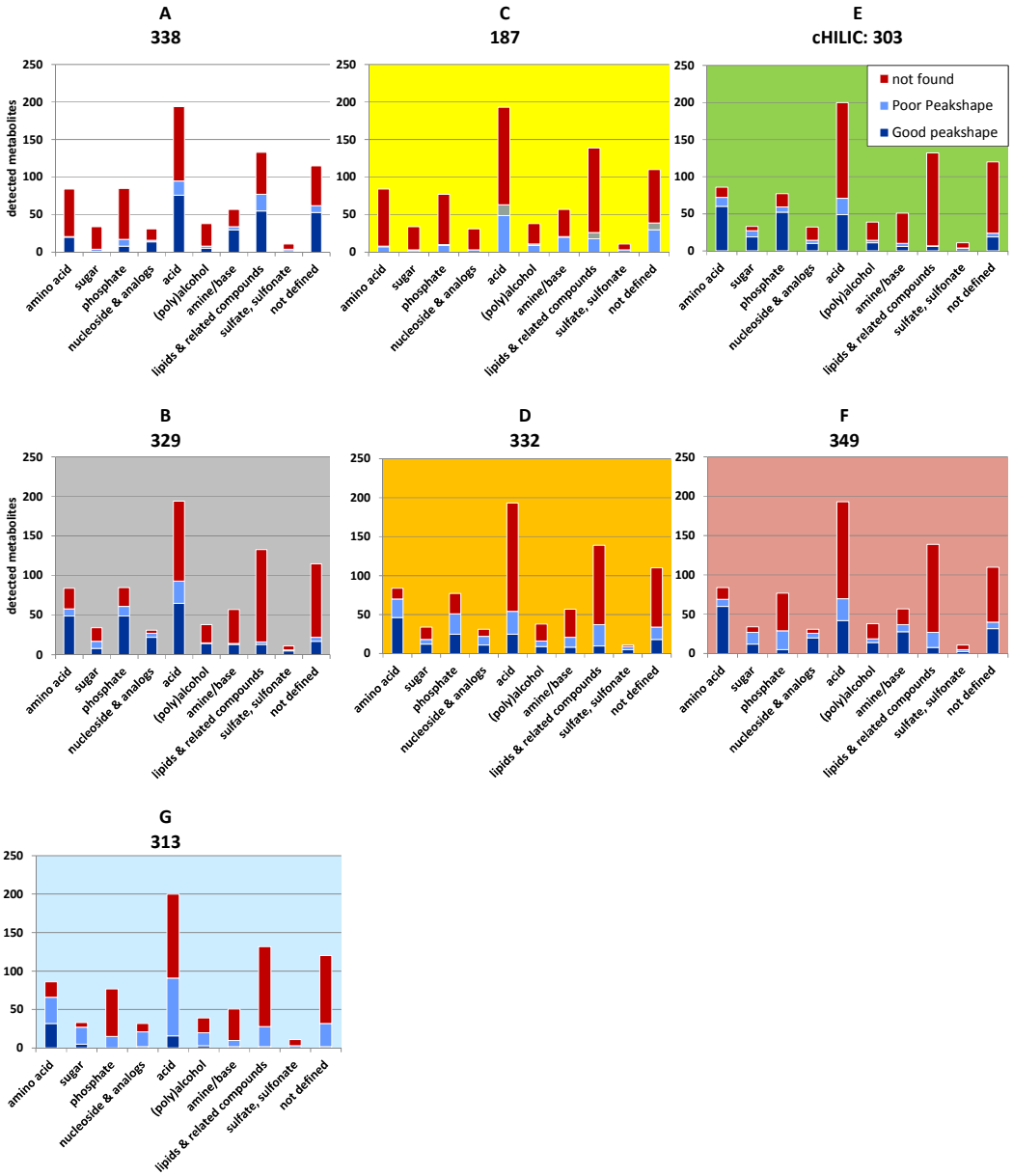

Fig. S3 Metabolite subgroups and their retention on columns A-G. The numbers on top of each panel denote total number of identified metabolites from the library of 714. Dark blue segments signify good and light blue segments signify poor peakshape (double/multiple, split, broad or tailing peaks). Missing fraction is shown as red segments. See Table S1 for method description and Supporting Information II (xlsx file) for full list of library compound classification and results