

Supplementary Material

Chemometric evaluation of hydrophilic interaction liquid chromatography stationary phases: resolving complex mixtures of metabolites

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Table S1. Full factorial experimental design carried out in this work.

Experimental design					Exp. ID	Column	pH	Ionic strength (mM)	Organic co-solvent
Exp. ID	Column	pH	Ionic strength (mM)	Organic co-solvent					
1	A	3	5	Acetonitrile	46	C	5.5	5	Methanol
2	A	3	25	Acetonitrile	47	C	5.5	25	Methanol
3	A	3	50	Acetonitrile	48	C	5.5	50	Methanol
4	A	3	5	Methanol	49	C	7	5	Acetonitrile
5	A	3	25	Methanol	50	C	7	25	Acetonitrile
6	A	3	50	Methanol	51	C	7	50	Acetonitrile
7	A	5.5	5	Acetonitrile	52	C	7	5	Methanol
8	A	5.5	25	Acetonitrile	53	C	7	25	Methanol
9	A	5.5	50	Acetonitrile	54	C	7	50	Methanol
10	A	5.5	5	Methanol	55	D	3	5	Acetonitrile
11	A	5.5	25	Methanol	56	D	3	25	Acetonitrile
12	A	5.5	50	Methanol	57	D	3	50	Acetonitrile
13	A	7	5	Acetonitrile	58	D	3	5	Methanol
14	A	7	25	Acetonitrile	59	D	3	25	Methanol
15	A	7	50	Acetonitrile	60	D	3	50	Methanol
16	A	7	5	Methanol	61	D	5.5	5	Acetonitrile
17	A	7	25	Methanol	62	D	5.5	25	Acetonitrile
18	A	7	50	Methanol	63	D	5.5	50	Acetonitrile
19	B	3	5	Acetonitrile	64	D	5.5	5	Methanol
20	B	3	25	Acetonitrile	65	D	5.5	25	Methanol
21	B	3	50	Acetonitrile	66	D	5.5	50	Methanol
22	B	3	5	Methanol	67	D	7	5	Acetonitrile
23	B	3	25	Methanol	68	D	7	25	Acetonitrile
24	B	3	50	Methanol	69	D	7	50	Acetonitrile
25	B	5.5	5	Acetonitrile	70	D	7	5	Methanol
26	B	5.5	25	Acetonitrile	71	D	7	25	Methanol
27	B	5.5	50	Acetonitrile	72	D	7	50	Methanol
28	B	5.5	5	Methanol	73	E	3	5	Acetonitrile
29	B	5.5	25	Methanol	74	E	3	25	Acetonitrile
30	B	5.5	50	Methanol	75	E	3	50	Acetonitrile
31	B	7	5	Acetonitrile	76	E	3	5	Methanol
32	B	7	25	Acetonitrile	77	E	3	25	Methanol
33	B	7	50	Acetonitrile	78	E	3	50	Methanol
34	B	7	5	Methanol	79	E	5.5	5	Acetonitrile
35	B	7	25	Methanol	80	E	5.5	25	Acetonitrile
36	B	7	50	Methanol	81	E	5.5	50	Acetonitrile
37	C	3	5	Acetonitrile	82	E	5.5	5	Methanol
38	C	3	25	Acetonitrile	83	E	5.5	25	Methanol
39	C	3	50	Acetonitrile	84	E	5.5	50	Methanol
40	C	3	5	Methanol	85	E	7	5	Acetonitrile
41	C	3	25	Methanol	86	E	7	25	Acetonitrile
42	C	3	50	Methanol	87	E	7	50	Acetonitrile
43	C	5.5	5	Acetonitrile	88	E	7	5	Methanol
44	C	5.5	25	Acetonitrile	89	E	7	25	Methanol
					90	E	7	50	Methanol

Column code as in Table 1 (A: XBridge™ Amide; B: TSK Gel Amide-80; C: Luna-NH₂; D: ZIC-HILIC; E: Acclaim™ Mixed-Mode HILIC-1). pH values: 3 - acid, 5.5 - moderately acid, 7 – neutral.

Table S2. MCR-ALS augmented matrices covering the different chromatographic conditions (same pH, ionic strength and organic co-solvent of the mobile phase) for the different stationary phases considered.

MCR-ALS analysis number*	Column		
	pH	Ionic strength (mM)	Organic co-solvent
1	3	5	Acetonitrile
2	3	5	Methanol
3	3	25	Acetonitrile
4	3	25	Methanol
5	3	50	Acetonitrile
6	3	50	Methanol
7	5.5	5	Acetonitrile
8	5.5	5	Methanol
9	5.5	25	Acetonitrile
10	5.5	25	Methanol
11	5.5	50	Acetonitrile
12	5.5	50	Methanol
13	7	5	Acetonitrile
14	7	5	Methanol
15	7	25	Acetonitrile
16	7	25	Methanol
17	7	50	Acetonitrile
18	7	50	Methanol

*Every MCR-ALS augmented data matrix contains experiments carried out using the five different HILIC columns in the same mobile phase conditions. For instance, MCR-ALS analysis number 1 contained experiments number 1 (XBridge™ Amide), 19 (TSK Gel Amide-80), 37 (Luna-NH₂), 55 (ZIC-HILIC) and 73 (Acclaim™ Mixed-Mode HILIC-1) of Table S1. All these runs were carried out using a mobile phase at pH 3.0 with an ionic strength of 5 mM and acetonitrile as organic co-solvent.

Table S3. Retention times of the MCR-ALS resolved elution profiles of the 11 metabolites for the 90 studied experimental conditions.

Exp. ID	Retention time (min)										Red. Glutathione
	Uridine	L-tryptophan	L-tyrosine	Phenylalanine	Inosine	Hypoxanthine	Cytidine	NADH	AMP	L-citrulline	
1	35.07	37.74	37.73	39.87	37.1	34.94	39.25	45.1	42.97	39.82	39.87
2	35.46	37.75	37.75	40.25	37.53	35.3	39.17	46.04	35.36	40.29	40.22
3	35.25	39.46	37.61	39.32	37.42	35.09	38.99	37.47	34.99	39.32	39.44
4	17.69	17.13	17.78	9.17	18.35	16.46	19.64	12.91	15.86	18.07	11.95
5	17.56	14.46	16.69	16.52	16.5	16.34	17.58	13.23	19.77	19.91	12.47
6	17.4	13.65	16.6	16.78	13.7	16.25	17.26	15.23	18.17	16.7	13.52
7	35.42	37.87	37.86	42.44	37.37	35.29	38.57	42.45	40.81	36.77	41.85
8	35.13	37.84	37.83	39.15	37.22	35.05	38.4	44.7	43.92	37.58	37.23
9	35.4	37.92	37.92	38.94	37.45	35.33	38.63	45.38	38.85	38.93	40.23
10	8.94	16.9	17.04	15.92	12.6	16.78	15.28	8.42	12.31	12.35	17.02
11	12.68	13.84	16.63	16.71	12.52	13.67	12.62	10.32	12.67	16.71	16.62
12	14.39	16.37	12.59	12.42	16.42	16.19	13.09	14.48	14.43	12.42	14.73
13	34.61	37.69	37.69	37.02	36.77	34.53	38	41.63	39.21	38.28	41.63
14	34.87	37.72	37.71	38.81	37.02	34.82	38.2	44.31	37.07	37.88	44.31
15	35.03	37.86	37.85	39.32	37.25	35.01	38.4	45.25	44.76	39.38	40.14
16	12.68	13.86	16.63	12.58	12.53	13.68	12.59	12.71	14.61	16.66	17.1
17	11.94	16.92	17.09	14.47	15.68	15.32	16.76	14.67	11.93	17.15	9.55
18	16.54	7.3	7.91	7.96	11.22	16.57	11.27	16.37	16.76	16.74	7.95
19	20.39	23.98	23.99	24.09	22.59	20.63	25.5	28.51	28.12	27.92	28.52
20	28.29	23.92	23.92	23.79	22.53	20.26	23.57	28.29	27.58	23.82	28.29
21	29.7	25.25	23.67	24.94	22.76	20.66	24.33	29.71	24.83	24.52	25.02
22	6.75	6.92	6.17	6.24	6.99	6.32	6.63	-	6.37	6.06	9.13
23	9.51	6.49	9.51	7.03	9.62	9.02	9.37	8.46	6.98	8.52	8.46
24	6.75	4.64	6.53	4.66	6.3	6.26	6.66	5.9	6.63	4.66	4.62
25	20.36	23.87	23.87	24.03	22.57	20.6	24.26	27.25	25.66	22.08	25.31
26	21.18	23.89	23.89	23.85	23.09	21.23	24.04	28.33	27.88	22.75	23.05
27	28.98	24.01	23.51	24.05	22.87	20.87	23.86	29.01	28.9	25.4	25.77
28	6.75	6.61	6.75	6	6.28	6.34	6.82	-	6.28	6.28	6.08
29	6.76	4.61	4.69	3.22	5.86	6.3	4.76	4.74	4.71	3.22	4.77
30	6.75	4.59	6.51	4.37	4.8	6.3	6.61	5.19	5.1	4.4	4.8
31	19.59	23.76	23.77	23.63	22.16	19.95	23.88	26.65	25.53	23.89	26.65
32	28.29	23.91	23.93	23.81	22.53	20.39	23.57	28.29	27.58	23.83	28.29
33	21.23	23.91	23.91	24.28	23.22	21.39	24.16	29.03	28.94	23.91	25.6
34	6.63	6.81	6.71	6.1	6.18	6.28	6.71	-	5.59	6.71	7.66
35	6.67	4.62	6.71	4.64	4.62	6.29	6.75	-	6.32	4.66	4.66
36	6.89	4.6	6.76	4.37	4.61	6.39	6.73	5.22	6.3	7.06	6.89
37	22.92	25.41	25.41	24.94	24.4	22.92	24.34	-	24.6	25.94	28.94
38	21.69	43.02	25.43	24.55	24.62	22.15	25.08	21.94	24.52	24.47	24.58
39	20.84	40.72	24.78	31.22	23.59	21.71	24.64	21.73	23.65	24.4	31.22
40	6.6	11.63	11.67	10.8	11.01	9.97	7.65	-	11.35	8.5	10.49
41	6.58	11.26	11.28	10.58	10.34	9.44	7.77	5.35	8.29	5.76	10.34
42	6.43	5.48	10.56	9.88	5.62	8.31	7.73	4.69	6.28	9.83	5.05
43	21.26	25.62	25.62	25.45	26.06	24.79	24.71	22.52	25.22	24.88	24.08
44	23.71	25.01	25.01	24.65	24.95	23.72	24.52	23.23	23.31	27.87	24.96

45	23.48	25	24.99	24.6	25.49	23.5	24.27	22.87	23.6	24.87	26.81
46	6.6	11.91	12.56	9.96	12.4	9.17	7.32	6.05	9.98	12.48	12.56
47	6.55	11.52	11.51	8.44	5.08	9.06	8.01	5.89	8.52	4.53	5.04
48	6.49	9.93	10.88	8.11	10.06	9.15	7.58	5.8	6.03	5.47	5.05
49	22.51	25.28	25.29	24.88	24.17	22.52	24.17	21.48	24.86	24.98	28.7
50	24.93	25.33	25.33	25.47	24.58	24.93	24.05	25.1	24.6	25.26	25.12
51	25.17	25.09	25.09	24.74	22.98	24.03	24.25	24.11	24.06	23.62	26.97
52	7.49	9.09	10.16	7.31	8.33	7.49	6.41	8.54	6.88	5.17	6.39
53	6.51	5.51	11.32	8.47	10.26	9.26	5.56	5.63	6.02	8.57	11.64
54	6.62	9.69	11.06	3.95	5.09	7.54	8.64	5.55	7.58	7.5	3.95
55	19.87	23.68	23.68	23.1	21.75	19.6	26.41	26.95	26.79	29.09	26.97
56	20.21	25.1	23.36	25.49	22.11	20.08	23.54	27.93	25.37	25.51	26.23
57	26.53	25.1	23.2	25.03	21.8	24.91	23.98	27.99	24.94	24.99	24.74
58	7.12	9.36	9.31	8.98	6.99	7.16	9.39	-	8.13	8.93	8.99
59	5.25	5.65	8.27	5.57	5.65	5.72	5.77	5.82	5.38	5.39	5.57
60	9.07	5.49	7.95	5.47	5.54	6.94	5.21	8.17	6.23	5.4	5.5
61	24.92	23.79	23.8	22.46	22.66	20.88	23.64	25.25	24.92	24	25.25
62	26.77	23.26	23.31	23.23	21.84	21.83	22.81	26.91	26.8	27.33	21.83
63	27.64	23.42	23.42	25.1	22.16	19.99	23.24	27.6	27.72	25.1	25.16
64	3.42	5.58	7.84	5.46	5.62	6.91	5.57	4.28	3.43	7.81	7.85
65	4.17	5.28	7.82	6.76	6.93	7.04	8.59	5.27	5.3	5.32	5.3
66	5.37	7.9	7.85	5.95	6.08	7.04	8.54	11.87	5.35	6.16	5.23
67	24.27	23.31	24.2	23.3	21.86	19.66	22.89	24.36	22.76	23.23	24.56
68	26.75	23.26	23.28	23.14	21.81	21.84	22.77	26.8	26.71	23.16	26.83
69	27.87	23.69	23.69	25.34	22.49	22.51	23.52	27.76	20.53	25.31	25.44
70	3.34	5.65	7.73	3.34	5.67	6.88	5.23	4.45	3.31	8.48	4.44
71	5.31	5.64	7.75	5.32	5.7	5.74	5.74	5.27	5.34	7.54	5.17
72	5.42	6.34	7.9	8.24	11.16	7.14	8.87	-	5.43	8.03	8.11
73	15.36	15.26	15.4	15.25	5.82	7.61	7.95	15.94	15.42	14.86	15.26
74	16.14	15.48	15.48	15.6	7.77	7.71	15.48	5.79	7.85	15.54	15.58
75	15.89	15.09	15.14	15.27	15.48	15.99	8.05	14.04	6.86	15.16	15.56
76	3.87	3.26	3.44	3.69	4.22	3.91	3.91	3.87	3.5	3.38	3.24
77	3.58	3.95	3.95	3.46	4.24	3.95	3.95	3.08	3.64	3.65	3.27
78	3.24	3.95	3.34	3.4	3.95	3.34	3.93	4.35	3.64	3.6	3.96
79	5.32	15.65	15.68	15.07	5.77	5.31	8.8	14.61	15.26	13.28	12.07
80	16.42	15.66	15.21	3.36	3.79	5.56	8.3	15.77	16.39	16.31	3.78
81	16.82	15.64	15.65	15.22	7.95	7.95	8.19	8	15.81	14.6	15.14
82	2.39	3.24	3.98	2.41	4.28	3.99	2.45	2.43	2.35	4.03	3.98
83	2.65	2.88	3.93	2.65	2.89	3.94	2.79	2.77	3.83	4	2.71
84	3.18	3.4	3.91	2.67	4.23	3.41	3.91	3.59	3.14	3.99	3.43
85	5.21	15.52	15.54	6.22	5.91	7.57	7.92	15.46	15.14	7.36	8.76
86	16.5	15.54	15.58	15	7.81	7.71	8.02	7.77	16.5	15.73	7.77
87	15.68	14.97	15.07	14.93	7.86	7.84	8.06	7.85	15.64	15.08	14.94
88	2.3	3.28	3.46	3.62	3.91	3.86	3.84	2.34	3.53	3.47	2.33
89	2.55	3.32	3.9	2.7	3.65	3.49	3.44	2.81	3.09	3.44	3.36
90	3.14	3.38	3.69	3.41	3.64	3.72	3.31	-	3.5	2.86	3.56

Figure S1. PCA models results. PC2 vs. PC1 scores plot considering: (A) all samples classified by organic co-solvent: acetonitrile samples (red diamonds) and methanol samples (green squares); (B) methanol samples classified by stationary phase: (BEH Amide (cyan triangles, ▼), Amide (blue triangles, ▲), Amine (red diamonds), Diol (green squares) and Zwitterionic (black stars); (C) acetonitrile samples classified by stationary phase (colors as in (B)); (D) acetonitrile samples after diol samples removal classified by stationary phase (colors as in (B)).

