

SUPPORTING INFORMATION

Optimizing Separations in On-line Comprehensive Two-dimensional Liquid Chromatography

Review

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S-1 Separation of industrial surfactant mixture

For the separation of industrial surfactants as shown in Figure 2 of the review. A Shimadzu LC20 setup was used. The system consisted of a CBM-20A communications bus module which controlled the system, four LC20AD pumps, a SIL-20AC autosampler, a DGU-20A degasser and a CTO-20A column oven. Inside the column oven a two-position Cheminert 10-port valve by Valco Instruments Co. Inc. and was located and configured for comprehensive operation.

Dimension 1

Column: Acclaim™ Surfactant Plus (250 mm × 2.1 mm, particle size: 3 μm). Gradient analysis. Oven temperature: 40°C. Flowrate: 10 μL min⁻¹ at 0.0 min, 80 μL min⁻¹ at 0.1 min, 80 μL min⁻¹ at 6.2 min, 10 μL min⁻¹ at 6.3 min. Analysis time: 201 minutes. Mobile phase: acetonitrile (A) and 0.1 M ammonium acetate in water with pH 5.4 through addition of glacial acetic acid (B). Gradient: 60% B at 0.0 min, 20% B at 130 min, 20% B at 140.0 min, 60% B at 150.0 min, 60% B at 201 min. Injection volume: 20 μL.

Dimension 2

Column: Phenomenex Kinetex C18 (50 mm × 4.6 mm, particle size: 5 μm). Gradient analysis. Oven temperature: 40 °C, flowrate: 3.5 mL min⁻¹, analysis time: 3 minutes. Mobile phase: water/acetonitrile [95:5] (A) and acetonitrile/water [95:5] (B). Gradient: 0% B at 0 sec, 100% B at 135 sec, 0% B at 165 sec, 0% at 180 sec.

S-2 Tailored Shifting Gradient

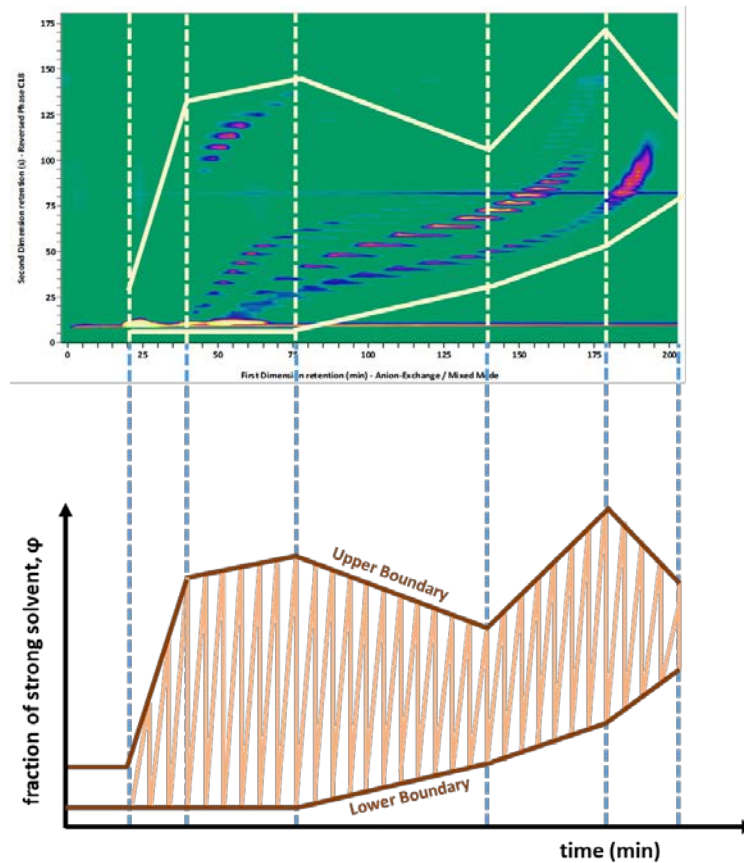


Figure S-1 – Example of a tailored shifting gradient program proposed to optimize the use of the separation space. Note that all the boundary lines connect to avoid peaks being split between dimensions.

S-3 Separation of mixture of sulfonated ethoxylates

Agilent PL-SAX (150 mm × 2.1 mm, particle size: 8 μm). Isocratic analysis. Oven temperature: 40 $^{\circ}\text{C}$, Flowrate: 1.5 mL min^{-1} , Analysis time: 60 minutes. Mobile phase: 10 mM ammonium acetate in water (A), 10 mM ammonium acetate in acetonitrile/water [1:1] (B). Injection volume: 10 μL .

S-5 Pareto-optimality plot

The scanning experiments comprised the separation of a mixture of aged, synthetic dyes by strong anion-exchange and ion-pair reversed-phase chromatography [41] using scouting conditions as reported by Pirok and co-workers [42].

To establish the retention parameters of all selected analytes of interest, the adsorption model [43] was used to fit the ion-exchange data and the linear solvent-strength model [44] was used to fit the reversed-phase data. The MATLAB-based PIOTR program (Program for Interpretive Optimization of Two-dimensional Resolution) used the FMINSEARCH function of MATLAB to solve the two integrated gradient equations for two unknown parameters in each dimension to determine the retention parameters for each compound. The resulting retention parameters are shown in Table S-2. For the ion-exchange separation, it can be seen that unrealistic retention parameters were found for analytes 1 to 14. This can be explained from the absence of retention for these analytes under ion-exchange conditions. The model failed at describing the non-existent retention behaviour. Moreover, for the retained analytes 36 and 28, unrealistic parameters were found in ion-exchange and reversed-phase LC, respectively. This shows that it is prudent to inspect the obtained retention parameters and improve or discard them if necessary.

Table 1 also lists the Akaike Information Criterion (AIC) [45] values for each compound. This goodness-of-fit value provides an indication on how well the used retention model is able to describe the retention behaviour. These goodness-of-fit values were earlier obtained by using five experimental datapoints per compound instead of two. Generally, lower values reflect a better ability of the retention model to describe the retention. These, and other statistical indications may help to assess the performance of the used retention model, as was also shown for the assessment of retention models to describe HILIC retention by Cesla and co-workers [46].

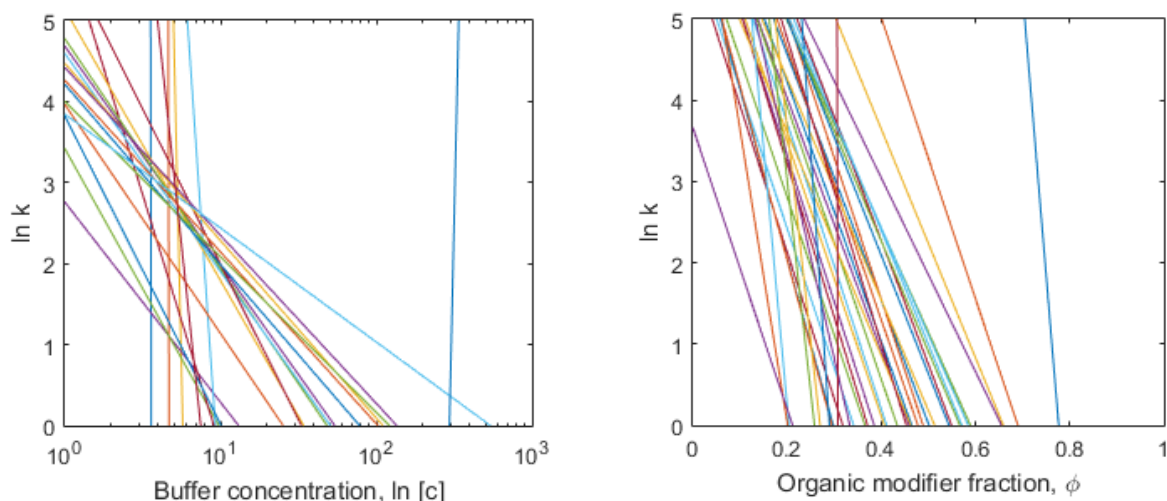


Figure S-2 – Established retention plots of 34 analytes for ion-exchange (left) using the adsorption model and ion-pair reversed-phase (right) using the linear solvent-strength model.

Table S-2 – Overview of fitted retention parameters all analytes of interest in both dimensions.

Analyte	<i>ION-EXCHANGE</i>			<i>REVERSED-PHASE</i>		
	$\ln k_0$	n	AIC	$\ln k_0$	S	AIC
1	-207.25	-36.44	17.44	53.88	69.39	-61.99
2	-207.25	-36.44	17.62	11.94	17.31	-52.45
3	-207.25	-36.44	16.79	9.28	14.08	-51.21
4	-207.25	-36.44	13.83	7.81	11.93	-48.64
5	-207.25	-36.44	13.37	7.56	12.83	-50.66
6	-207.25	-36.44	13.23	8.00	14.01	-52.00
7	-207.25	-36.44	16.32	8.37	15.24	-49.16
8	-207.25	-36.44	15.25	7.60	15.16	-53.49
9	-207.25	-36.44	17.35	8.53	17.46	-52.51
10	-207.25	-36.44	14.58	6.88	16.91	-50.64
11	-207.25	-36.44	16.78	6.91	17.95	-53.62
12	-207.25	-36.44	13.30	6.21	17.01	-54.76
13	-207.25	-36.44	13.71	5.87	17.18	-56.34
14	-207.25	-36.44	18.31	5.74	18.05	-54.03
17	63.34	36.30	-53.40	7.00	13.63	-50.44
18	2.76	1.07	-28.14	7.14	15.57	-51.19
19	3.42	1.50	-30.63	7.29	16.82	-53.03
20	26.94	12.10	-42.41	7.25	17.63	-53.21
21	5.97	2.71	-33.82	6.99	18.85	-51.20
22	3.82	1.65	-31.10	6.23	20.90	-53.45
23	3.96	1.23	-25.57	6.32	21.39	-54.18
24	5.14	1.46	-25.73	6.36	13.75	-49.90
25	4.68	1.17	-22.72	8.31	24.93	-55.09
26	4.78	1.23	-27.62	13.43	52.02	-60.94
27	4.59	1.17	-26.67	12.95	63.45	-62.43
28	5.83	1.67	-25.61	838.51	2722.60	-1.84
29	4.21	0.97	-25.03	24.73	85.32	-64.01
30	4.26	0.92	-23.62	7.25	36.02	-56.10
31	4.47	0.95	-25.15	14.16	52.41	-60.45
32	4.42	0.90	-22.61	3.66	17.30	-52.14
33	4.00	0.83	-23.53	7.71	13.60	-50.70
34	3.85	0.61	-16.02	7.70	13.18	-53.50
35	15.77	7.84	-42.20	8.53	18.81	-50.67
36	-207.25	-36.44	14.14	7.92	14.56	-51.02

Using the obtained retention parameters, potential LCxLC methods were generated by varying several method parameters as is reflected in Table S-3. The total number of simulated LCxLC methods was thus 10,368 (product of all options).

Table S-3 – Optimization factors, ranges and values used for optimization.

Method Parameter	Min. Value	Max. Value	Increments	# Options
<i>¹D initial buffer conc. [c]_{init} (mM)</i>	0	10	5	3
<i>¹D final buffer conc. [c]_{final} (mM)</i>	25	100	25	4
<i>¹D gradient time (min)</i>	40	150	10	12
<i>²D initial modifier fraction φ_{init}</i>	0.05	0.3	0.05	6
<i>²D initial modifier fraction φ_{final}</i>	0.7	0.95	0.05	6
<i>²D gradient time (min)</i>	1.3	1.5	0.2	2

The retention times were predicted for each of the generated methods and the quality descriptor resolution was calculated according to Schure's metric for resolution in two-dimensional chromatography [47]. The resolutions between all the peaks were summarized into a Deringer-based score [42]. The resulting resolution values were then plotted against the analysis time (time for the last analyte to elute) in the Pareto-optimality plot as shown below.

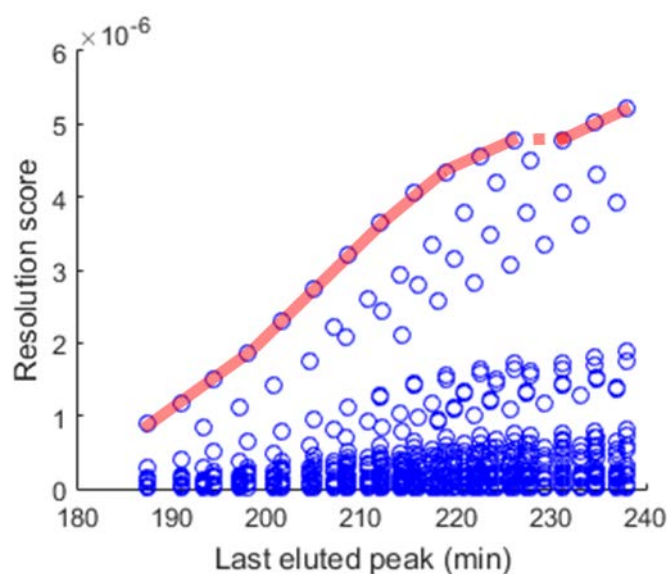


Figure S-3 – Pareto-optimality plot comprising the resolution plotted against the analysis time for 10368 simulated LCxLC methods.

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