Supporting information for : Rapid Method Development in Hydrophilic Interaction Liquid Chromatography for Pharmaceutical Analysis Using a Combination of Quantitative Structure-Retention Relationships and Design of Experiments

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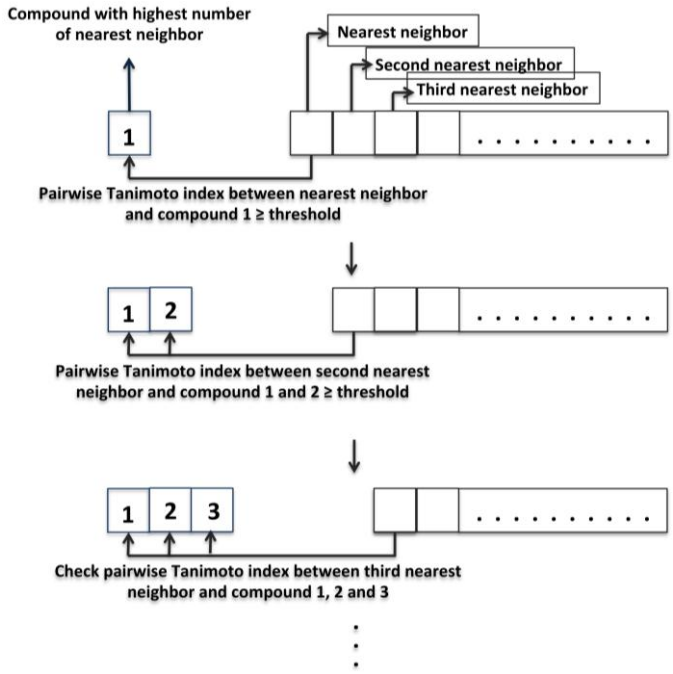

Figure S1. Scheme of in-house clustering algorithm.

Table S1. Plan of experiments defined by central composite design.

nr.	Acentonitrile content in mobile phase	pH	Salt concentration in mobile phase
1	70	3	10
2	90	3	10
3	70	7	10
4	90	7	10
5	70	3	20
6	90	3	20
7	70	7	20
8	90	7	20
9	70	5	15
10	90	5	15
11	80	3	15
12	80	7	15
13	80	5	10
14	80	5	20
15	80	5	15
16	80	5	15
17	80	5	15

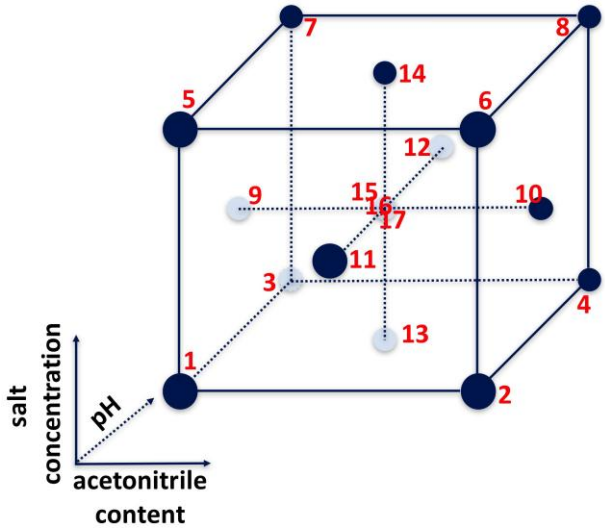

Figure S2. Diagram of the design space.

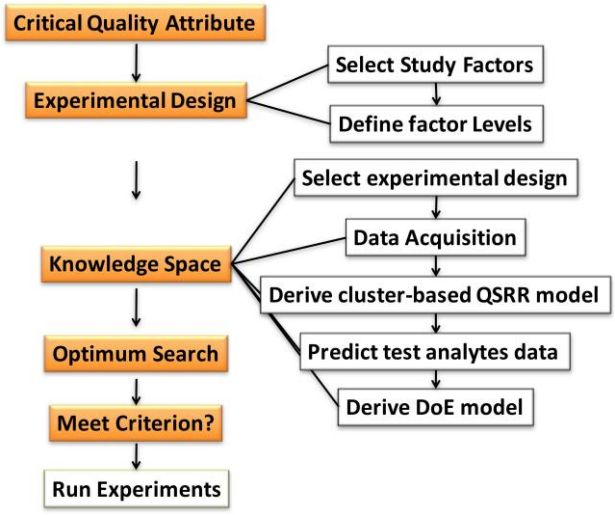

Figure S3. Scheme of QbD workflow followed in this work.

Table S2. Experimentally obtained retention times for each operating condition of the central composite design.

nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.92	5.98	2.39	6.55	2.17	6.16	2.47	7.19	2.33	6.64	2.78	3.21	2.63	3.15	3.07	3.04	3.07
2	1.93	6.40	2.44	7.20	2.21	6.66	2.54	8.45	2.36	7.20	2.86	3.33	3.13	3.24	3.15	3.15	3.15
3	1.91	6.75	2.33	6.83	2.15	6.83	2.41	7.42	2.30	6.85	2.79	3.13	3.00	3.07	3.01	2.98	3.03
4	1.91	5.88	2.32	6.25	2.15	5.98	2.40	6.70	2.28	6.22	2.74	3.10	2.96	3.04	2.97	2.95	2.99
5	1.93	6.49	2.39	6.99	2.20	6.68	2.48	7.62	2.35	6.99	2.86	3.29	3.12	3.22	3.13	3.11	3.14
6	1.94	6.41	2.37	6.86	2.18	6.62	2.46	7.46	2.33	6.88	2.84	3.24	3.08	3.18	3.09	3.07	3.11
7	2.02	7.64	2.57	8.38	2.28	8.16	2.61	9.54	2.48	8.60	3.09	3.52	3.43	3.49	3.41	3.39	3.42
8	1.81	3.98	2.25	4.34	2.04	3.89	2.31	4.40	2.19	4.26	2.43	2.79	2.66	2.71	2.65	2.64	2.68
9	1.99	7.58	2.48	8.12	2.25	7.99	2.57	9.06	2.41	8.23	3.02	3.51	3.31	3.43	3.34	3.31	3.35
10	1.94	6.06	2.41	6.75	2.18	6.24	2.50	7.28	2.35	6.73	2.82	3.30	3.11	3.21	3.12	3.10	3.14
11	1.92	6.26	2.39	6.90	2.17	6.46	2.47	7.60	2.33	6.98	2.79	3.25	3.07	3.17	3.09	3.07	3.10
12	1.98	7.55	2.49	8.24	2.26	7.97	2.58	9.18	2.43	8.31	3.04	3.55	3.35	3.47	3.37	3.35	3.38
13	1.99	7.93	2.50	8.63	2.26	8.40	2.59	9.75	2.44	8.78	3.06	3.58	3.38	3.50	3.40	3.38	3.41
14	1.86	5.04	2.27	5.39	2.09	5.01	2.34	5.67	2.23	5.33	2.58	2.92	2.79	2.86	2.80	2.79	2.82
15	1.76	3.98	2.10	3.46	1.97	3.84	2.15	3.51	2.08	3.48	2.32	2.45	2.42	2.45	2.42	2.42	2.44
16	4.02	3.70	3.13	3.60	3.09	3.51	2.80	3.60	3.06	3.50	3.05	2.88	3.15	2.88	3.09	3.18	2.97
17	3.71	3.58	3.09	3.57	2.93	3.36	2.78	3.54	3.02	3.45	2.89	2.83	3.10	2.84	3.04	3.13	2.93
18	2.19	2.75	4.06	6.27	2.17	2.70	3.59	6.19	3.31	5.41	2.25	3.96	3.89	3.54	3.91	3.85	3.62
19	4.62	4.71	3.46	4.54	3.49	4.54	3.11	4.50	3.36	4.39	3.57	3.27	3.62	3.29	3.57	3.69	3.38
20	3.51	4.60	3.44	5.18	2.89	4.29	3.09	5.14	3.35	4.99	3.04	3.38	3.73	3.38	3.67	3.77	3.49
21	2.43	3.78	3.88	9.69	2.32	3.69	3.45	9.80	3.55	8.54	2.50	4.31	4.61	4.13	4.55	4.34	4.25
22	2.29	2.68	4.05	6.00	2.19	2.54	3.57	5.64	3.58	5.15	2.21	3.84	4.14	3.61	4.11	3.94	3.74
23	2.31	3.04	4.39	7.69	2.27	3.03	3.88	7.63	3.50	6.41	2.40	4.48	4.34	3.92	4.38	4.06	4.02
24	2.33	2.82	4.34	7.51	2.24	2.72	3.82	7.25	3.76	6.25	2.31	4.40	4.60	4.09	4.61	4.32	4.20
25	2.22	2.76	4.18	6.71	2.19	2.69	3.71	6.55	3.48	5.59	2.27	4.13	4.17	3.72	4.18	3.91	3.83
26	2.30	2.89	4.26	7.57	2.23	2.80	3.78	7.33	3.66	6.29	2.31	4.35	4.50	4.01	4.50	4.20	4.13
27	2.32	2.69	4.67	7.63	2.22	2.56	4.06	7.27	3.97	6.17	2.23	4.48	4.76	4.14	4.80	4.47	4.29
28	2.12	2.39	3.85	5.17	2.07	2.35	3.42	5.02	3.31	4.46	2.11	3.55	3.67	3.29	3.68	3.48	3.40
29	2.54	3.98	2.55	3.96	2.55	4.03	2.57	4.07	2.56	3.95	2.96	2.95	2.95	2.98	2.95	2.94	2.90
30	2.87	8.70	2.87	8.51	2.87	9.74	2.90	9.79	2.87	8.81	3.87	3.86	3.86	3.93	3.88	3.86	3.78
31	2.46	4.16	2.45	4.13	2.46	4.58	2.48	4.52	2.46	4.27	2.89	2.88	2.88	2.93	2.90	2.89	2.85
32	2.68	5.46	2.67	5.42	2.68	5.99	2.71	5.93	2.68	5.55	3.35	3.33	3.33	3.40	3.35	3.34	3.28
33	2.99	10.91	2.99	10.70	2.99	12.84	3.03	12.67	2.99	11.22	4.26	4.26	4.24	4.34	4.28	4.25	4.15
34	2.72	6.91	2.73	6.91	2.73	8.12	2.76	8.09	2.73	7.29	3.57	3.59	3.56	3.65	3.59	3.58	3.51
35	2.38	3.53	2.37	3.51	2.38	3.77	2.39	3.73	2.38	3.58	2.71	2.70	2.69	2.73	2.70	2.70	2.67
36	2.48	4.30	2.47	4.27	2.48	4.85	2.50	4.76	2.48	4.46	2.95	2.95	2.93	3.00	2.96	2.95	2.91
37	2.78	7.60	2.79	7.18	2.80	8.18	2.82	8.77	2.80	7.58	3.66	3.70	3.65	3.71	3.70	3.73	3.64
38	2.28	2.95	2.29	2.90	2.29	2.97	2.30	3.01	2.29	2.92	2.48	2.48	2.48	2.49	2.49	2.52	2.47

Numbers in row are operating conditions of the central composite design (Table S1). Numbering (in column) of compounds: 1, 3-methoxytyramine; 2, isoproterenol; 3, fenotrole; 4, terbutaline; 5, salbutamol; 6, metaproterenol; 7, dopamine; 8, N-methylephedrine; 9, norfenefrine; 10, phenylephrine; 11, tyramine; 12, normetanephrine; 13, octopamine; 14, methoxamine; 15, isoxuprine; 16, salicylic acid; 17, 5-methylsalicylic acid; 18, 4-hydroxybenzoic acid; 19, 2,3-dihydroxybenzoic acid; 20, 2,4-dihydroxybenzoic acid; 21, 3,5-dihydroxybenzoic acid; 22, benzoic acid; 23, 3-amino-4-hydroxybenzoic acid; 24, 3-aminobenzoic acid; 25, vanillic acid; 26, syringic acid; 27, 2-methoxybenzoic acid; 28, p-toluic acid; 29, 2',3'-dideoxyadenosine; 30, 3'-deoxyguanosine; 31, 5-methyluridine; 32, adenosine; 33, guanosine; 34, inosine; 35, thymidine; 36, uridine; 37, acyclovir; 38, 3'-deoxythymidine.

Table S3. Coefficients of the obtained DoE models for training set and their statistical evaluation.

	β_0(p)	β_1(p)	β_2(p)	β_3(p)	β_4(p)	β_5(p)	β_6(p)	β_7(p)	β_8(p)	Q^2	R^2	R^2 adj.
1	2.997 (0.000)	2.124 (0.000)	0.280 (0.000)	0.168 (0.002)	1.383 (0.000)		0.103 (0.048)			0.989	0.997	0.995
2	3.150 (0.000)	2.525 (0.000)	0.473 (0.000)	0.116 (0.048)	1.672 (0.000)		0.323 (0.000)			0.984	0.996	0.995
3	3.006 (0.000)	2.358 (0.000)	0.169 (0.000)	0.107 (0.006)	1.573 (0.000)					0.995	0.998	0.998
4	2.970 (0.000)	1.996 (0.000)	0.211 (0.000)	0.095 (0.002)	1.240 (0.000)					0.995	0.999	0.998
5	3.128 (0.000)	2.342 (0.000)	0.261 (0.000)	0.130 (0.003)	1.486 (0.000)		0.087 (0.039)			0.993	0.998	0.997
6	3.090 (0.000)	2.295 (0.000)	0.240 (0.000)	0.124 (0.002)	1.460 (0.000)					0.994	0.998	0.997
7	3.400 (0.000)	3.037 (0.000)	0.342 (0.000)	0.204 (0.013)	2.030 (0.000)					0.983	0.995	0.993
8	2.656 (0.000)	1.027 (0.000)	0.193 (0.000)		0.491 (0.000)					0.99	0.996	0.994
9	3.330 (0.000)	2.930 (0.000)	0.291 (0.000)	0.183 (0.005)	1.937 (0.000)					0.991	0.997	0.995
10	3.120 (0.000)	2.168 (0.000)	0.300 (0.000)	0.113 (0.004)	1.325 (0.000)		0.117 (0.007)			0.993	0.998	0.997
11	3.081 (0.000)	2.291 (0.000)	0.300 (0.000)	0.133 (0.008)	1.467 (0.000)		0.125 (0.019)			0.99	0.997	0.996
12	3.362 (0.000)	2.951 (0.000)	0.324 (0.000)	0.185 (0.004)	1.938 (0.000)		0.133 (0.036)			0.99	0.997	0.996
13	3.393 (0.000)	3.170 (0.000)	0.342 (0.000)	0.207 (0.006)	2.133 (0.000)		0.151 (0.047)			0.987	0.997	0.995
14	2.798 (0.000)	1.566 (0.000)	0.200 (0.000)	0.062 (0.021)	0.924 (0.000)					0.994	0.998	0.997
15	2.418 (0.000)	0.820 (0.000)			0.415 (0.000)					0.965	0.988	0.982
16	3.010 (0.000)	0.181 (0.000)	- 0.137(0.002)	- 0.172(0.000)	0.392 (0.000)		0.147 (0.002)	0.131 (0.004)	0.100 (0.019)	0.630	0.955	0.920
17	2.946 (0.000)	0.197 (0.000)	- 0.066(0.024)	- 0.162(0.000)	0.358 (0.000)		0.118 (0.002)	0.106 (0.004)	0.082 (0.014)	0.786	0.966	0.939
18	3.745 (0.000)	0.800 (0.000)	1.201 (0.000)		0.624 (0.001)	- 0.630(0.001)	0.464 (0.000)			0.925	0.973	0.96
19	3.460 (0.000)	0.464 (0.000)	- 0.205(0.002)	- 0.202(0.002)	0.613 (0.000)		0.168 (0.010)	0.158 (0.014)		0.684	0.963	0.935
20	3.542 (0.000)	0.792 (0.000)	0.190 (0.000)	- 0.166(0.000)	0.708 (0.001)	- 0.252(0.001)	0.163 (0.001)	0.079 (0.028)	0.068 (0.049)	0.977	0.994	0.988
21	4.422 (0.000)	1.986 (0.000)	1.640 (0.000)		1.557 (0.000)	- 1.083(0.002)	1.180 (0.000)			0.926	0.974	0.962
22	3.885 (0.000)	0.633 (0.000)	1.120 (0.000)	- 0.161(0.039)	0.532 (0.001)	- 0.810(0.000)	0.410 (0.000)			0.933	0.978	0.965
23	4.170 (0.000)	1.144 (0.000)	1.500 (0.000)		0.810 (0.001)	- 0.706(0.002)	0.695 (0.000)			0.931	0.975	0.964
24	4.364 (0.000)	1.005 (0.000)	1.491 (0.000)		0.697 (0.002)	- 0.948(0.000)	0.704 (0.000)			0.93	0.974	0.962
25	3.976 (0.000)	0.852 (0.000)	1.316 (0.000)		0.611 (0.002)	- 0.723(0.001)	0.541 (0.000)			0.929	0.973	0.961
26	4.275 (0.000)	1.064 (0.000)	1.476 (0.000)		0.750 (0.001)	- 0.893(0.000)	0.712 (0.000)			0.932	0.975	0.963
27	4.470 (0.000)	0.910 (0.000)	1.610 (0.000)		0.698 (0.005)	- 1.015(0.000)	0.682 (0.000)			0.919	0.969	0.955
28	3.501 (0.000)	0.463 (0.000)	1.000 (0.000)		0.422 (0.004)	- 0.633(0.000)	0.297 (0.002)			0.919	0.969	0.955
29	2.950 (0.000)	0.722 (0.000)		0.023 (0.011)	0.330 (0.000)					0.998	0.999	0.998
30	3.863	3.118		0.243	2.130			0.286		0.996	0.998	0.997

	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)			
31	2.890 (0.000)	0.934 (0.000)	0.090 (0.000)	0.508 (0.000)	0.098 (0.000)	0.996	0.998	0.998
32	3.340 (0.000)	1.493 (0.000)	0.115 (0.000)	0.838 (0.000)	0.126 (0.000)	0.997	0.998	0.998
33	4.256 (0.000)	4.334 (0.000)	0.404 (0.000)	3.080 (0.000)	0.482 (0.000)	0.995	0.997	0.996
34	3.580 (0.000)	2.367 (0.000)	0.252 (0.000)	1.520 (0.000)	0.293 (0.000)	0.996	0.998	0.997
35	2.700 (0.000)	0.623 (0.000)	0.051(0.000)	0.303 (0.000)	0.054 (0.000)	0.996	0.998	0.998
36	2.950 (0.000)	1.024 (0.000)	0.114 (0.000)	0.554 (0.000)	0.126 (0.000)	0.995	0.998	0.997
37	3.684 (0.000)	2.533 (0.000)	0.228 (0.003)	1.646 (0.000)	0.265 (0.003)	0.985	0.994	0.992
38	2.487 (0.000)	0.330 (0.000)	0.017 (0.024)	0.133 (0.000)		0.99	0.996	0.994

Numbering (in column) of compounds: 1, 3-methoxytyramine; 2, isoproterenol; 3, fenotrole; 4, terbutaline; 5, salbutamol; 6, metaproterenol; 7, dopamine; 8, N-methylephedrine; 9, norfenefrine; 10, phenylephrine; 11, tyramine; 12, normetanephrine; 13, octopamine; 14, methoxamine; 15, isoxuprine; 16, salicylic acid; 17, 5-methylsalicylic acid; 18, 4-hydroxybenzoic acid; 19, 2,3-dihydroxybenzoic acid; 20, 2,4-dihydroxybenzoic acid; 21, 3,5-dihydroxybenzoic acid; 22, benzoic acid; 23, 3-amino-4-hydroxybenzoic acid; 24, 3-aminobenzoic acid; 25, vanillic acid; 26, syringic acid; 27, 2-methoxybenzoic acid; 28, p-toluic acid; 29, 2',3'-dideoxyadenosine; 30, 3'-deoxyguanosine; 31, 5-methyluridine; 32, adenosine; 33, guanosine; 34, inosine; 35, thymidine; 36, uridine; 37, acyclovir; 38, 3'-deoxythymidine. DoE model is $t_R = \beta_0 + \beta_1 \times$ acetonitrile content $+ \beta_2 \times$ pH $+ \beta_3 \times$ salt concentration $+ \beta_4 \times$ (acetonitrile content)$^2 + \beta_5 \times$ (pH)$^2 + \beta_6 \times$ (acetonitrile content $\times$ pH) $+ \beta_7 \times$ (acetonitrile content $\times$ salt concentration) $+ \beta_8 \times$ (acetonitrile content $\times$ pH). p is the significance of the variables in the model.

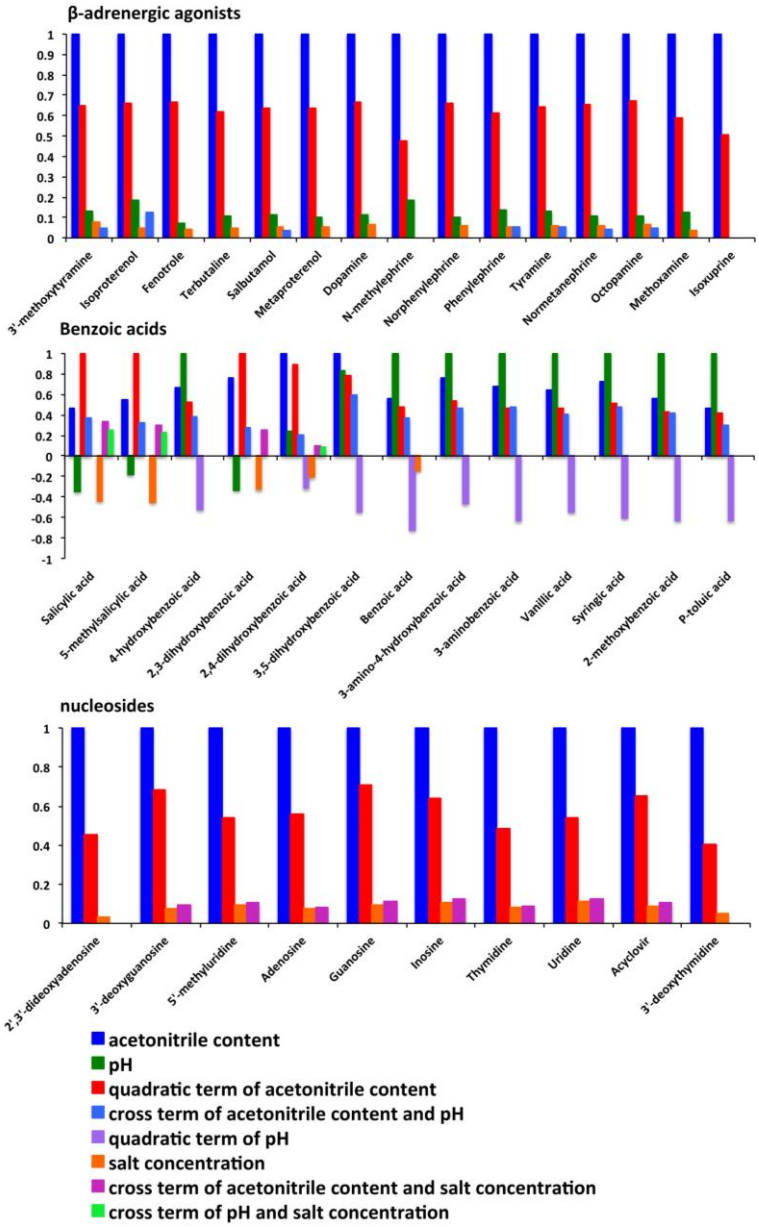

Figure S4. DoE model term ranking chart for each compound in the training set. In model term ranking, the variable that has the highest coefficient value is assigned a ranking of 1, and other variables are ranked based on the experimental data. The data indicate that acetonitrile content (and its quadratic term) was the most significant factor in determining retention of β-adrenergic agonists and nucleosides in agreement with the understanding that hydrophilic partitioning is vitally important in the mechanism of retention for HILIC.

For benzoic acids with $\boldsymbol{pKa > 4}$, the pH ranked even higher than the acetonitrile content, while possibly supporting the same mechanism, hydrophilic partitioning. A possible explanation of a large positive coefficient of pH is the increased hydrophilicity of benzoic acids, due to solute protonation in higher pH.[1,2] The term pH in DoE models obtained for benzoic acids with $\boldsymbol{pKa < 3}$, however, has a negative coefficient, indicating a moderate and negative effect on their retention behavior. A possible explanation is that the amount of charged silanols increases by increasing the mobile phase pH, which might magnify the electrostatic repulsion with the negative charged analytes eroding the observed retention behavior.[1] In addition, it is also interesting to note the positive and small importance of the pH on β-adrenergic agonists. These results suggest that the electrostatic attraction between the positively charged bases and the negatively charged silanols on the surface of amide stationary phase is increased in higher pH.

Another interesting observation is a small and positive effect of salt concentration on β-adrenergic agonist and nucleoside retention, due possibly to the promotion of stronger hydrophilic partitioning in higher salt concentration.[3] A negative salt effect, however, was observed on some benzoic acids retention, suggesting that the decreased hydrophilicity of the solute, possible due to interaction of the ammonium counterion in the eluent with the negatively charged benzoic acids.

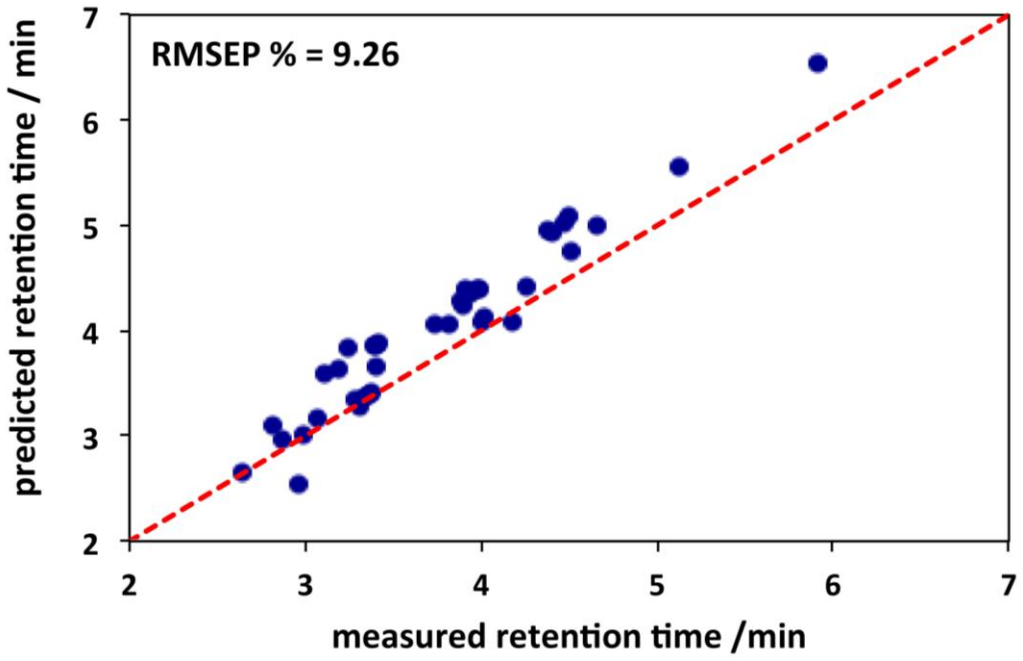

Figure S5. Experimental values versus predicted values of external validation of DoE models for analyzed compounds under a never analyzed chromatographic condition: 10 mmol L^{-1} ammonium formate (pH 4) containing 85% v/v acetonitrile.

Table S4. Summary of porting equations on the structurally-similarity based classified datasets.

dataset	porting equation
β-agonists	$t_{Rported} = 0.716\ t_{Rmeasured}^{a} + 0.872$
Benzoic acids	$t_{Rported} = 0.931\ t_{Rmeasured} + 0.015$
nucleosides	$t_{Rported} = 0.671\ t_{Rmeasured} + 1.100$

a is measured retention time when the column was new

Table S5. Predictive performance of GA-PLS models on internal validation.

	RMSECV / % in datasets:		
nr.	β-agonists	benzoic acids	nucleosides
1	0.43	5.83	0.64
2	3.50	8.61	4.40
3	0.64	12.01	0.63
4	5.71	38.05	6.01
5	0.59	3.79	0.87
6	4.74	6.59	6.46
7	0.52	5.70	0.83
8	3.93	34.91	4.88
9	0.64	9.88	0.66
10	3.51	22.13	4.26
11	1.22	3.56	0.96
12	1.07	11.47	1.78
13	2.81	19.55	2.12
14	1.39	8.84	1.49
15	1.44	16.58	1.24
16	1.02	12.84	1.63
17	1.09	15.35	1.42

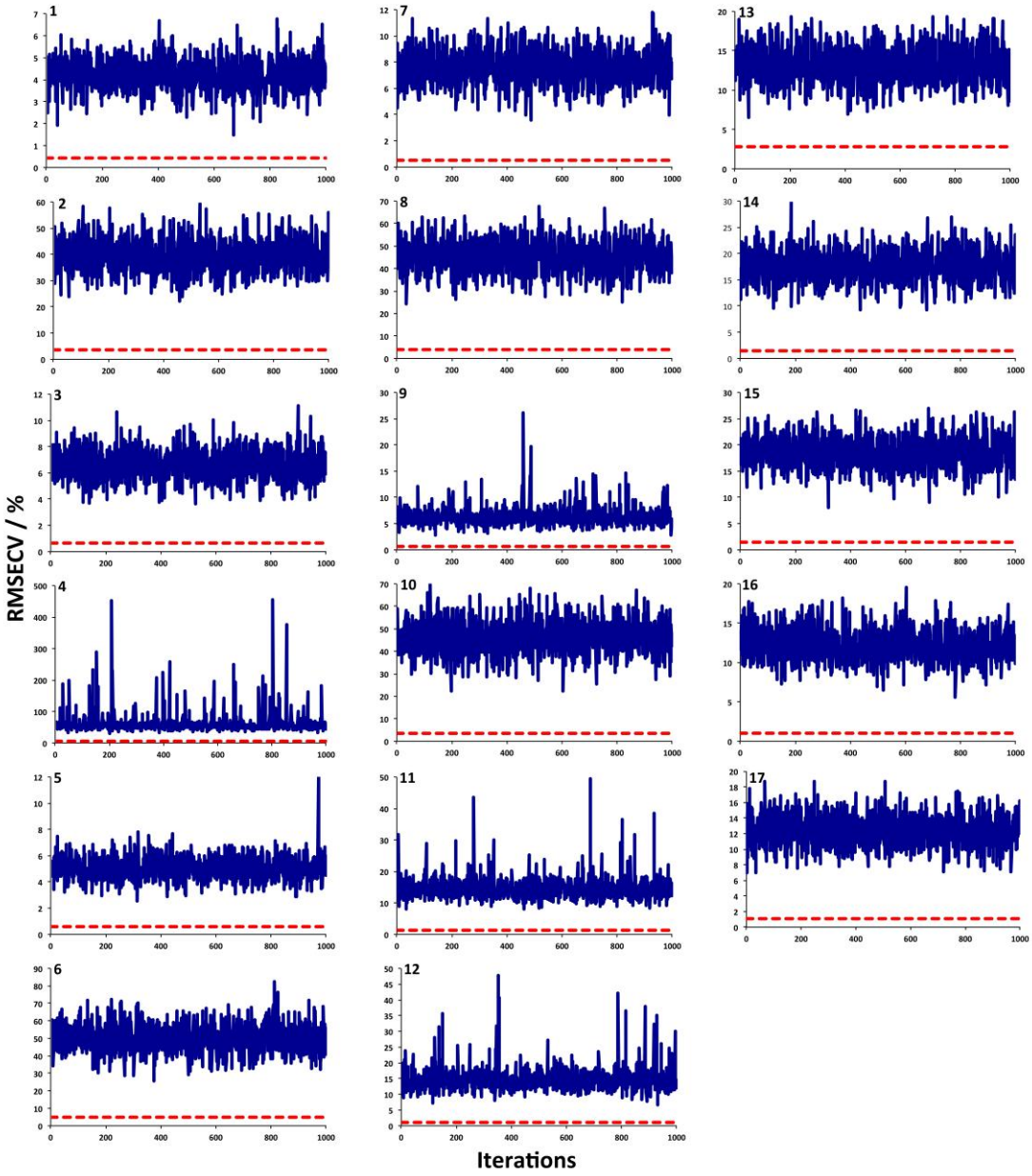

Figure S6. Y-randomization plots of β-adrenergic agonists dataset on 17 operating chromatographic conditions of the studied central composite design. Y-randomized data (royal blue lines), and the actual data (red line).

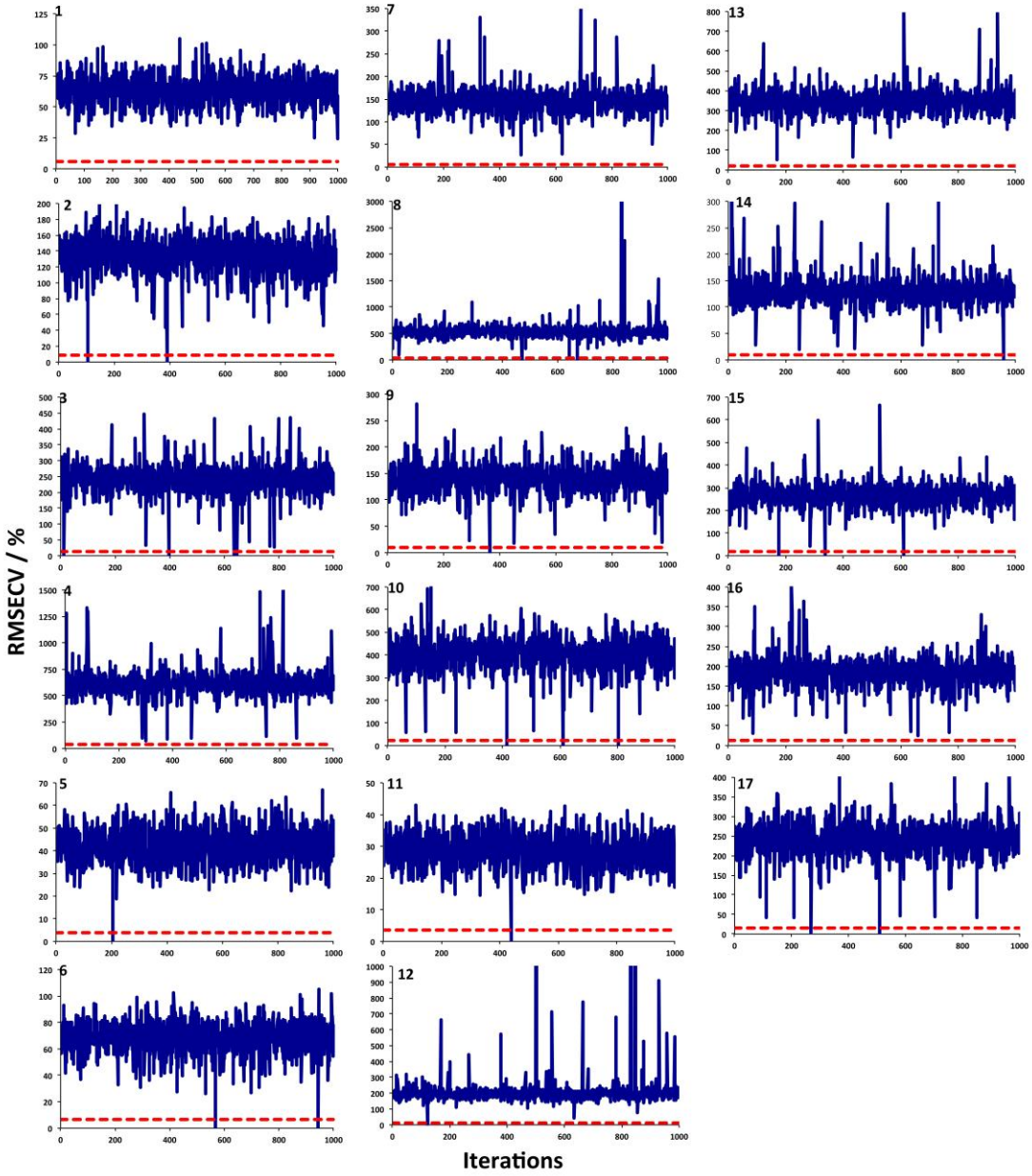

Figure S7. Y-randomization plots of benzoic acids dataset on 17 operating chromatographic conditions of the studied central composite design. Y-randomized data (royal blue lines), and the actual data (red line).

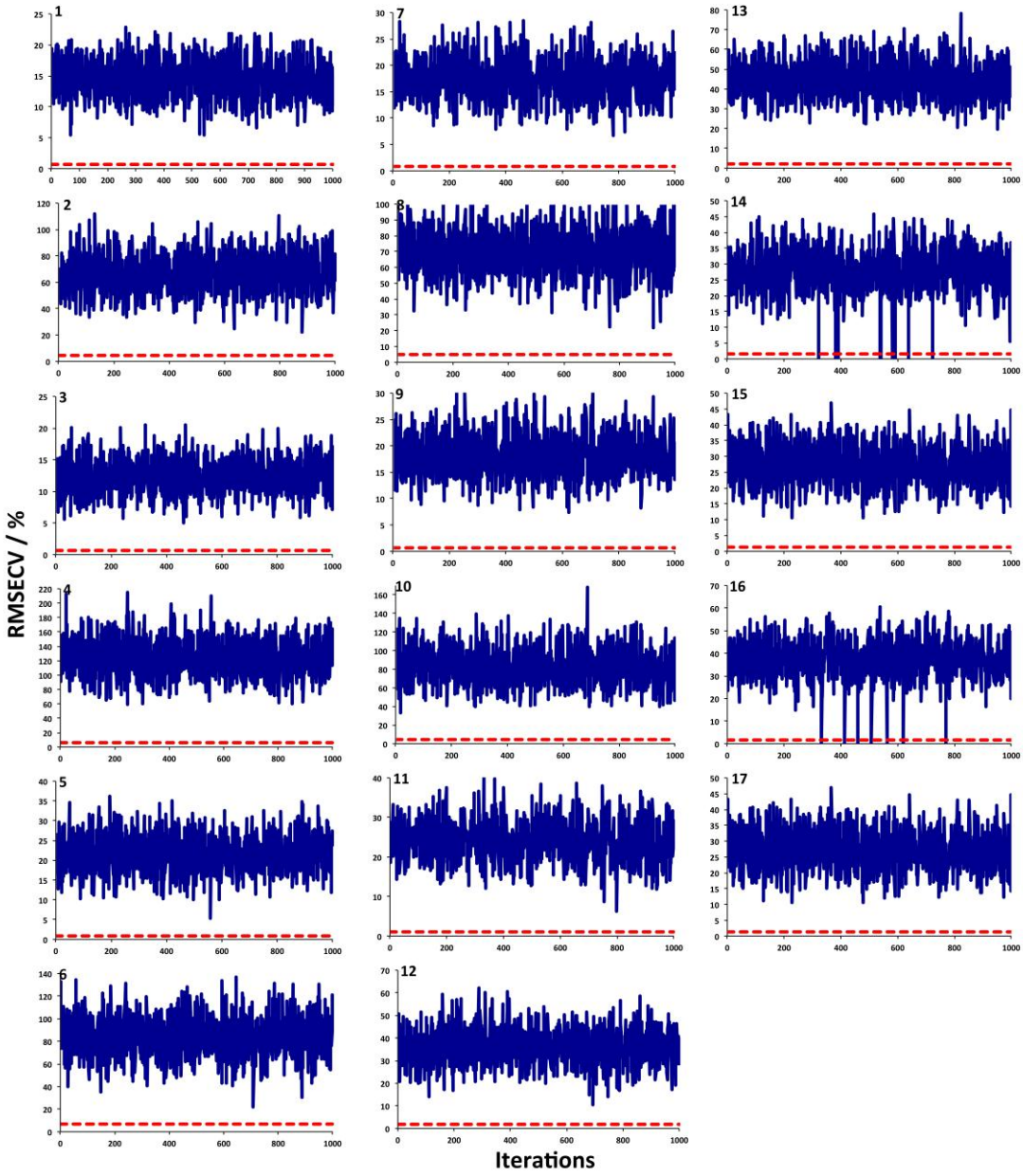

Figure S8. Y-randomization plots of nucleosides dataset on 17 operating chromatographic conditions of the studied central composite design. Y-randomized data (royal blue lines), and the actual data (red line).

Table S6. Coefficients of the obtained DoE models for test sets and their statistical evaluation.

analytes	β_0(p)	β_1(p)	β_2(p)	β_3(p)	β_4(p)	β_5(p)	β_6(p)	β_7(p)	Q^2	R^2	R^2 adj.
Adrenaline	3.418 (0.000)	3.190 (0.000)	0.447 (0.000)	0.209 (0.008)	2.148 (0.000)		0.268 (0.004)		0.986	0.996	0.995
Noradrenaline	3.674 (0.000)	3.960 (0.000)	0.436 (0.000)	0.259 (0.013)	2.748 (0.000)		0.238 (0.034)		0.984	0.996	0.994
Ritudrine	2.934 (0.000)	1.762 (0.000)			1.034 (0.000)		-0.245 (0.001)		0.975	0.993	0.989
Synephrine	3.200(0.000)	2.353 (0.000)	0.319 (0.000)	0.133 (0.019)	1.463 (0.000)		0.132 (0.033)		0.987	0.996	0.994
3-Hydroxybenzoic acid	4.123 (0.000)	1.270 (0.000)	1.346 (0.000)		1.139 (0.002)	-1.081 (0.003)	0.813 (0.001)		0.725	0.964	0.928
2,5-Dihydroxybenzoic acid	4.048 (0.000)	1.025 (0.000)			1.276 (0.000)	-0.913 (0.003)	0.413 (0.014)		0.674	0.941	0.883
4-Aminobenzoic acid	3.617 (0.000)	0.296 (0.004)	0.830 (0.000)			-0.603 (0.002)			0.776	0.955	0.91
4-Aminosalicylic acid	3.258 (0.000)	0.530 (0.010)	0.487 (0.010)		0.608 (0.010)				0.589	0.899	0.797
2'-deoxyadenosine	3.225 (0.000)	1.350 (0.000)		0.094 (0.001)	0.761 (0.000)			0.103 (0.001)	0.995	0.997	0.996
2'-deoxyguanosine	4.995 (0.000)	3.034 (0.000)							0.655	0.805	0.76
2'-deoxyuridine	3.226 (0.000)	1.050 (0.000)							0.713	0.843	0.807
2'-deoxyinosine	4.050 (0.000)	1.863 (0.000)							0.724	0.847	0.812

DoE model is $t_R = \beta_0 + \beta_1 \times$ acetonitrile content $+ \beta_2 \times$ pH $+ \beta_3 \times$ salt concentration $+ \beta_4 \times$ (acetonitrile content)^2 + $\beta_5 \times$ (pH)^2 + $\beta_6 \times$ (acetonitrile content × pH) + $\beta_7 \times$ (acetonitrile content × salt concentration) + $\beta_8 \times$ (acetonitrile content × pH). p is the significance of the variables in the model.

Table S7. The experimental and predicted retention times of test compounds for each operating condition of the central composite design by using GA-PLS models.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Adrenaline																	
$t_{Rmeasured}$	2.00	7.75	2.56	9.38	2.28	8.33	2.65	10.39	2.47	9.12	3.09	3.69	3.45	3.58	3.48	3.45	3.46
$t_{Rpredicted}$	2.00	7.79	2.54	8.98	2.28	8.26	2.62	10.11	2.44	8.64	3.07	3.63	3.40	3.53	3.43	3.42	3.43
Noradrenaline																	
$t_{Rmeasured}$	2.05	9.81	2.63	12.26	2.35	10.86	2.73	13.45	2.54	11.32	3.32	3.96	3.70	3.85	3.75	3.71	3.71
$t_{Rpredicted}$	2.06	9.28	2.64	10.32	2.35	9.99	2.71	11.80	2.55	10.52	3.30	3.86	3.75	3.79	3.67	3.65	3.69
Ritudrine																	
$t_{Rmeasured}$	1.84	5.43	2.25	5.66	2.08	5.37	2.33	6.01	2.22	5.63	2.57	2.90	2.78	2.84	2.79	2.76	2.81
$t_{Rpredicted}$	1.89	6.06	2.32	5.07	2.14	6.01	2.40	5.72	2.28	5.81	2.72	3.04	2.95	3.01	2.94	2.93	2.95
Synephrine																	
$t_{Rmeasured}$	1.94	6.32	2.43	7.10	2.19	6.53	2.52	7.74	2.36	7.15	2.85	3.36	3.16	3.27	3.17	3.15	3.18
$t_{Rpredicted}$	1.96	6.40	2.46	7.06	2.21	6.61	2.53	7.83	2.39	7.19	2.87	3.36	3.22	3.27	3.20	3.20	3.27
3-Hydroxybenzoic acid																	
$t_{Rmeasured}$	2.34	3.09	3.99	7.50	2.25	2.97	3.52	7.36	3.58	6.67	2.34	4.12	4.37	3.85	4.33	4.35	4.03
$t_{Rpredicted}$	2.34	3.15	3.92	7.42	2.25	2.95	3.42	7.93	3.71	6.88	2.35	3.81	4.31	3.59	4.22	4.38	4.04
2,5-Dihydroxybenzoic acid																	
$t_{Rmeasured}$	4.17	4.77	3.20	4.59	3.22	4.62	2.89	4.64	3.14	4.46	3.38	3.15	3.45	3.17	3.39	3.50	3.25
$t_{Rpredicted}$	4.32	4.80	3.27	5.58	3.34	4.80	2.98	5.91	3.93	7.00	3.43	3.12	4.06	4.02	4.10	3.56	4.22
4-Aminosalicylic acid																	
$t_{Rmeasured}$	2.80	3.59	3.66	5.40	2.50	3.32	3.27	5.24	3.52	5.01	2.58	3.58	3.96	3.55	3.89	3.91	3.68
$t_{Rpredicted}$	3.10	3.59	3.63	4.84	2.59	3.55	3.49	5.11	3.34	4.36	2.79	3.42	4.05	3.00	3.35	3.08	2.84
4-Aminobenzoic acid																	
$t_{Rmeasured}$	2.14	2.47	3.71	4.34	2.12	2.48	3.36	4.27	2.92	3.78	2.20	3.39	3.23	3.01	3.25	3.07	3.06
$t_{Rpredicted}$	2.15	2.67	3.80	4.74	2.15	2.62	3.79	4.38	3.47	3.92	2.27	3.45	3.82	3.81	3.52	3.45	3.79
2'-Deoxyadenosine																	
$t_{Rmeasured}$	2.60	4.73	2.60	4.71	2.60	4.93	2.63	4.99	2.61	4.75	3.16	3.15	3.14	3.19	3.16	3.15	3.09
$t_{Rpredicted}$	2.63	5.10	2.63	5.21	2.63	5.56	2.66	5.61	2.63	5.19	3.25	3.18	3.21	3.27	3.25	3.25	3.16
2'-Deoxyguanosine																	
$t_{Rmeasured}$	2.89	8.93	2.88	8.75	2.89	9.88	2.92	10.10	2.89	9.08	3.95	3.95	3.93	4.01	3.95	3.93	3.85
$t_{Rpredicted}$	2.91	8.26	2.82	8.01	2.86	10.03	2.87	10.02	2.85	8.33	3.76	3.60	4.01	3.85	3.52	3.51	3.71
2'-Deoxyuridine																	
$t_{Rmeasured}$	2.39	3.73	2.40	3.62	2.41	3.91	2.41	4.06	2.40	3.76	2.77	2.77	2.75	2.79	2.78	2.82	2.75
$t_{Rpredicted}$	2.45	4.48	2.42	3.97	2.44	5.23	2.45	4.49	2.42	4.49	2.87	2.86	2.88	2.85	2.83	2.88	2.84
2'-Deoxyinosine																	
$t_{Rmeasured}$	2.63	5.95	2.65	5.75	2.66	6.45	2.67	6.96	2.65	6.09	3.35	3.39	3.34	3.40	3.39	3.43	3.33
$t_{Rpredicted}$	2.66	5.94	2.65	6.06	2.67	6.80	2.66	7.20	2.65	5.93	3.43	3.37	3.40	3.34	3.34	3.37	3.39

Numbers in row are operating conditions of the central composite design (Table S1).

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