Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

L-lysine derived organogelator-based stationary phase for mixed-mode liquid chromatography

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1. Materials and methods

1.1 Materials

Sulfaquinoxaline and sulfamonomethoxine were obtained from Wako (Osaka, Japan). sulfamethoxazole, sulfamethizole, sulfanilamide and sulfathiazole were obtained from TCI (Tokyo, Japan). Sulfamerazine and sulfadiazine were obtained from Sigma (St Louis, MO, USA). All the analytes were used without further purification. HPLC grade solvents hexane, tetrahydrofuran and acetonitrile were purchased from Nacalai Tesque (Tokyo, Japan) whereas ethanol was purchased from Wako (Osaka, Japan). Ammonium acetate was purchased from Wako and acetic acid from Sigma-Aldrich. All nucleobases were commercially available and were used without further puriifacation.

1.2 Chromatographic Conditions

L-lysine-based organic phases on silica (Sil-Lys-Urea and Sil-Lys-Amide) stationary phases were packed into a stainless steel column (150 mm × 4.6 mm i.d.). YMC silica (YMC SIL-120-S5 having a 5 □m diameter, and a 12 nm pore size) was used. We used commercial amide column (Inertsil® Amide for HILIC separation from GL Sciences) (150 mm × 4.6 mm i.d.) packed with carbamoyl bonded spherical silica (5 μm diameter and 100 Å pore size) as one of the reference columns. HPLC-grade acetonitrile and Milipore water were used as components of the mobile phase for HILIC whereas HPLC grade Hexane, THF and Ethanol were used as mobile phase for normal phase chromatography. The HILIC mode measurements were carried out in a chromatographic system that included JASCO PU-2089 Plus pump and JASCO MD-910 multi-wavelength detector. 5 μL sample was injected through a Rheodyne model 7725 sample injector having 200 μL loop. On the other hand

normal phase measurements were carried out in a chromatographic system that included JASCO PU-980 pump, JASCO MD-2010 multi-wavelength detector and JASCO CD-2095 chiral detector. In this case, sample was injected through Rheodyne model 7725 injector having 20 μL loop. A personal computer connected to the detector and pump with ChromNAV (1.17 or later) software was used for system control and data analysis. Chromatographic grade solvent was used to prepare mobile phase solutions. The retention factor (k) was calculated using the equation:

$$k = \frac{(t_r - t_0)}{t_0} \quad (1)$$

Where, t_r is the retention time of the sample and t_0 is the void time. t_0 was calculated by injecting water in the case of HILIC mode. However, in case of normal phase chromatography t_0 was determined by noting the solvent peak position in the chromatogram for real samples.

Selectivity was given by:

$$\alpha = \frac{k_2}{k_1} \quad (2)$$

Where, k_2 is the retention factor for more strongly retained compound and k_2 is that for less strongly retained compound.

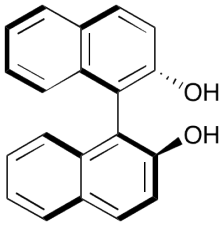

Fig. S1 Structure of 1, 1'-bi-2-napthol.

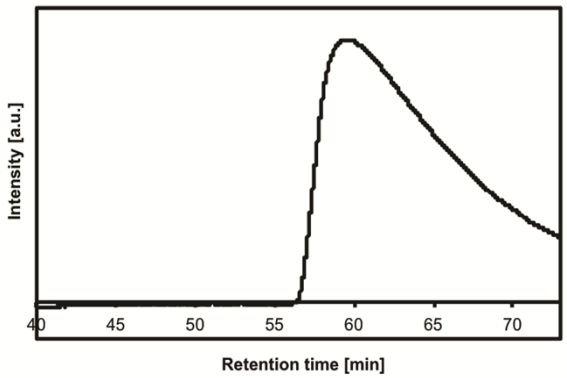

Fig. S2 Separation of mixture of *R(+)*- and *S(-)*- 1,1'-bi-2-napthol into its constituent isomers with Sil-Lys-Amide column as detected by UV detector. Mobile phase: hexane: tetrahydrofuran: ethanol; 70:30:0.5 at 0 ºC, flow rate 1 mL min^{-1}, λ= 254 nm.

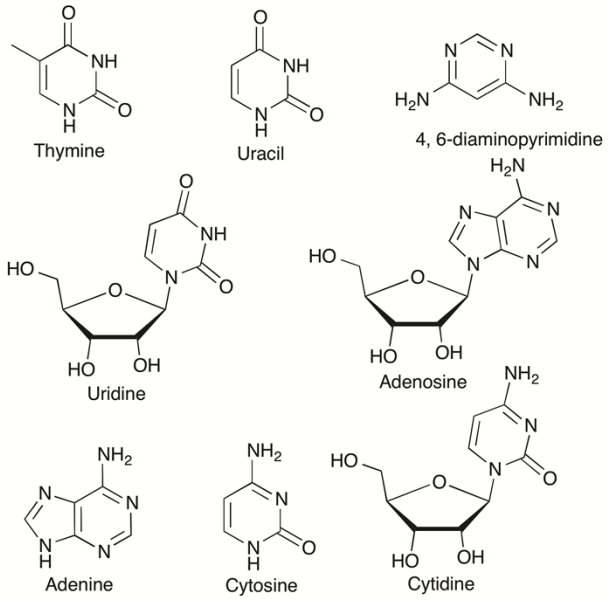

Fig. S3 Structure of nucleobase analytes.

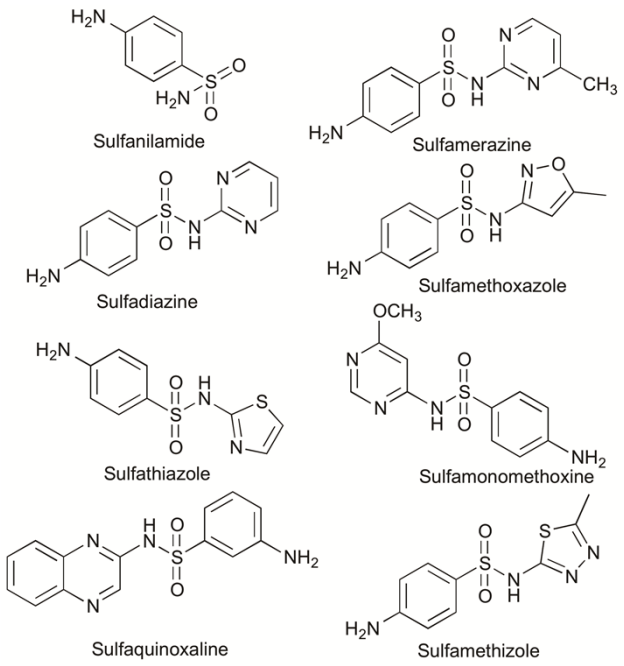

Fig. S4 Structures of sulfonamide antibiotic analytes.

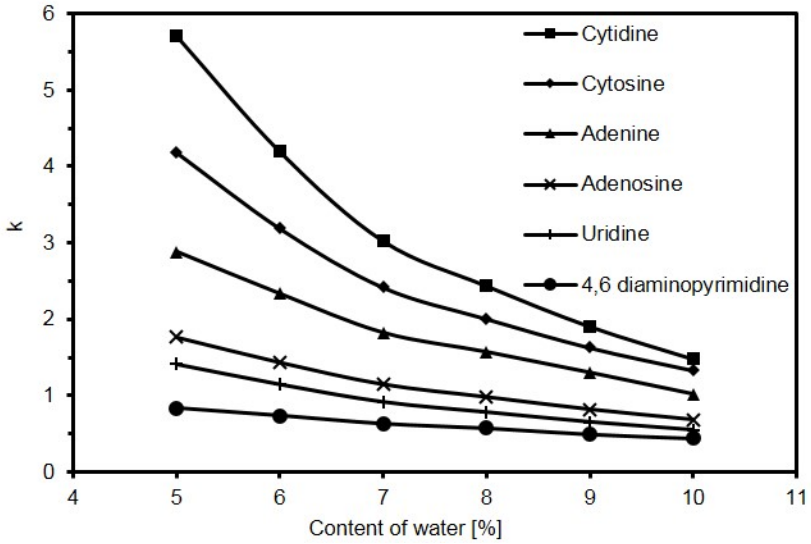

Fig. S5 Effect of water content on the retention behavior of some nucleobases with Sil-Lys-Urea column. Mobile phase is acetonitrile and ammonium acetate (20 mM, pH 6.7) with water content as indicated. Flow rate 1mL min^{-1}, *T*= 25 ˚C, λ= 254 nm.

Table S1 t_R, resolution (R_s), k, α and number of theoretical plates (N) of sulfur drugs for the Sil-Lys-Urea column at different buffer salt concentration. Mobile phase: acetonitrile: ammonium acetate (pH 6.7); 90:10, flow rate 1 mL min^{-1}, *T*= 25 ˚C, λ= 275 nm.

Sulfur Drugs	10 mM					15 mM					20 mM				
	t_R	R_s	k	α	N	t_R	R_s	k	α	N	t_R	R_s	k	α	N
Sulfurnilamide	1.867	4.429	0.037	-	1253	1.880	3.383	0.038	-	896	1.893	2.637	0.046	-	814
Sulfamerazine	2.627	5.636	0.459	12.394	6220	2.520	5.204	0.392	10.142	6191	2.400	4.446	0.325	7.082	6414
Sulfadiazine	3.493	4.956	0.940	2.048	6366	3.280	4.161	0.812	2.070	6343	3.000	3.528	0.657	2.016	6334
Sulfametho-xazole	4.507	5.035	1.503	1.598	5888	4.067	4.022	1.246	1.535	5758	3.613	3.579	0.996	1.515	5339
Sulfathiazole	5.773	6.641	2.207	1.467	7339	4.960	6.110	1.740	1.395	7376	4.333	5.670	1.394	1.399	7109
Sulfamono methoxine	7.987	4.376	3.437	1.557	6417	6.653	3.597	2.675	1.537	6715	5.720	3.296	2.160	1.549	6453
Sulfaquinoxa-line	9.827	21.677	4.459	1.297	7824	7.880	18.064	3.353	1.253	7700	6.693	16.100	2.697	1.248	7592
Sulfamethizole	25.893	-	13.385	3.001	9881	17.840	-	8.856	2.640	9138	14.227	-	6.860	2.542	8132

25 mM				
t_R	R_s	k	α	N
1.880	2.844	0.038	-	1337
2.333	3.757	0.289	7.475	6709
2.813	2.938	0.554	1.917	6259
3.293	4.186	0.819	1.478	5051
4.093	4.309	1.261	1.539	6847
5.067	2.955	1.799	1.426	6312
5.840	14.880	2.226	1.237	7507
12.42	-	5.865	2.634	6468

Table S2 t_R, R_s, k, α and N of nucleobases for the Sil-Lys-Urea column at different buffer salt concentration (for 20 mM buffer salt concentration please refer to Table S3 pH 6.7). Mobile phase: acetonitrile: ammonium acetate (pH 6.7); 95:5, flow rate 1 mL min^{-1}, *T*= 25 ˚C, λ= 254 nm.

Nucleobases	10 mM					15 mM					25mM				
	t_R	R_s	k	α	N	t_R	R_s	k	α	N	t_R	R_s	k	α	N
Thymine	3.04	1.19	0.51	-	2143	3.08	1.16	0.52	-	1979	3.04	1.10	0.49	-	1988
Uracil	3.34	1.61	0.66	1.30	2815	3.39	1.71	0.68	1.30	2819	3.32	1.76	0.63	1.28	3074
4, 6 diaminopyrimidine	3.79	3.42	0.88	1.33	2642	3.87	3.43	0.91	1.35	2523	3.80	3.29	0.86	1.37	2457
Uridine	5.04	1.68	1.51	1.70	2102	5.17	1.70	1.56	1.71	2055	5.04	1.65	1.47	1.70	2020
Adenosine	5.80	5.32	1.88	1.25	2457	5.97	5.13	1.96	1.25	2391	5.80	4.70	1.84	1.25	2366
Adenine	8.67	3.20	3.31	1.76	3194	8.82	3.72	3.37	1.72	3180	8.29	4.21	3.06	1.66	3192
Cytosine	10.76	4.33	4.35	1.31	3815	11.34	4.50	4.62	1.37	3834	11.00	4.39	4.39	1.43	3917
Cytidine	14.37	-	6.15	1.41	3471	15.32	-	6.58	1.43	3484	14.72	-	6.21	1.41	3483

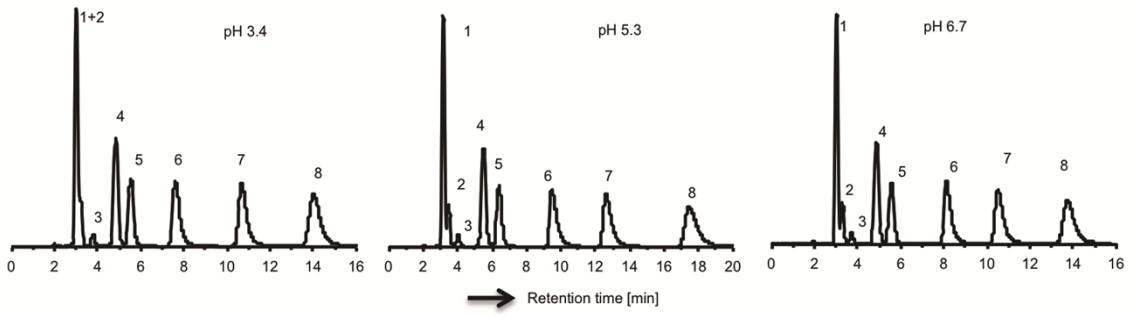

Fig. S6 Separation of nucleobases for the Sil-Lys-Urea column at different buffer pH. Mobile phase: acetonitrile: ammonium acetate (20 mM); 95:5, flow rate 1 mL min^{-1}, T= 25 ˚C, λ= 254 nm.

Table S3 t_R, R_s, k, α and N of nucleobases for the Sil-Lys-Urea column at different buffer pH. Mobile phase: acetonitrile: ammonium acetate (20 mM); 95:5, flow rate 1 mL min^{-1}, T= 25 ˚C, λ= 254 nm.

Nucleo-bases	pH 3.4					pH 5.3					pH 6.7				
	t_R	R_s	k	α	N	t_R	R_s	k	α	N	t_R	R_s	k	α	N
Thymine	3.01	2.65	0.48	-	1864	3.17	1.07	0.55	-	1758	3.01	1.15	0.48	-	2247
Uracil						3.48	1.81	0.71	1.27	2654	3.29	1.58	0.62	1.28	3177
4,6-diamino pyrimidine	3.79	2.89	0.86	1.79	2452	4.03	3.54	0.97	1.38	2297	3.71	3.31	0.83	1.33	2605
Uridine	4.85	1.51	1.38	1.61	2002	5.52	1.67	1.76	1.75	1880	4.88	1.58	1.40	1.70	2159
Adenosine	5.53	3.82	1.71	1.24	2236	6.4	4.84	2.14	1.25	2178	5.56	5.08	1.74	1.24	2516
Adenine	7.60	4.82	2.72	1.59	2433	9.47	4.01	3.64	1.7	2747	8.11	3.88	2.99	1.72	3327
Cytosine	10.67	4.27	4.23	1.55	4185	12.63	4.57	5.19	1.42	3482	10.51	4.08	4.18	1.39	3835
Cytidine	14.01	-	5.87	1.39	3778	17.45	-	7.55	1.45	3062	13.73	-	5.76	1.38	3660

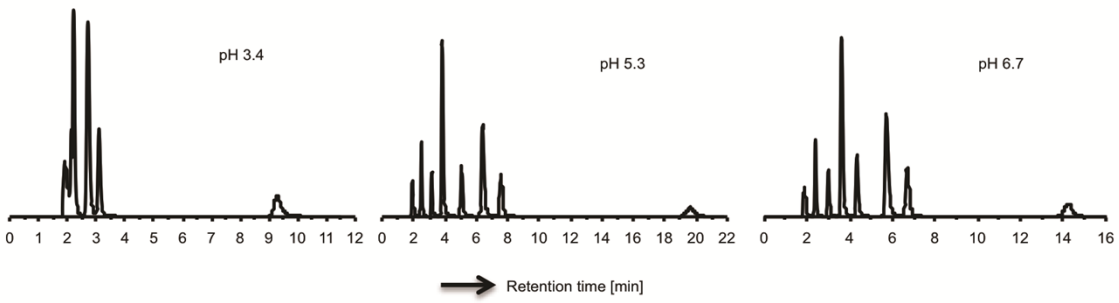

Fig. S7 Separation of sulfur based drugs for the Sil-Lys-Urea column at different buffer pH. Mobile phase: acetonitrile: ammonium acetate (20 mM); 90:10, flow rate 1 mL min^{-1}, T= 25 ˚C, λ= 275 nm.