Forming of a Novel Type of HPLC Column by Bridging of Discrete Silica Particles

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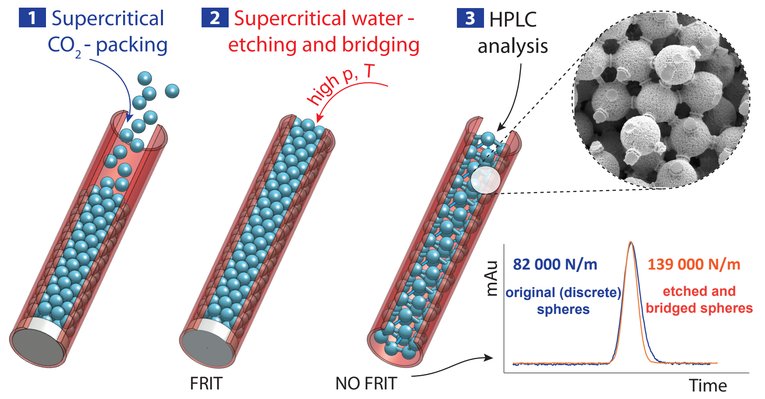
Abstract

A novel technique for producing highly uniform structures from silica microspheres has been developed and tested. It is based on exploiting the temperature- and pressure-dependent solvent properties of sub/supercritical water toward silicon dioxide. The initial concept aimed to create a "hybrid" capillary chromatographic column on the border between a packed and a monolithic column that would combine the benefits of both. The resultant method that integrates dissolution and coalescence in a continuous process enabled the production of a range of permeable columns with high efficiency and varying sizes. Their internal structures were examined using scanning electron microscopy and characterized using microHPLC chromatography. The structures produced using this method may have diverse applications beyond the scope of analytical chemistry. They prove useful in scenarios where high pressure is necessary because of the high hydraulic resistance of small particles and/or the passing medium with high flow rate. A simple test of a bridged-microsphere monolithic column and a discrete microsphere-packed column, both after chemical modification to C18 stationary phase, indicated superior performance of the new type of monolithic columns.

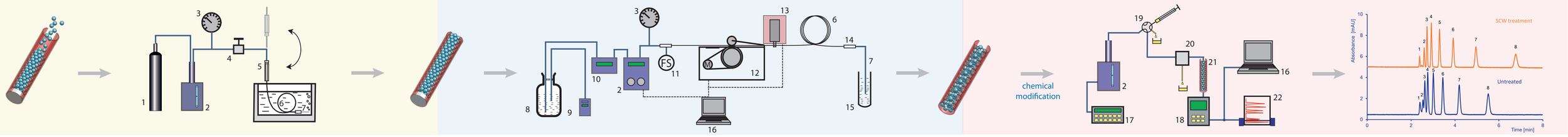


Instrumentation

Phase 1 - Supercritical CO_2 – packing
discrete 5µm non-porous silica spheres + 100µm i.d. fused silica capillary

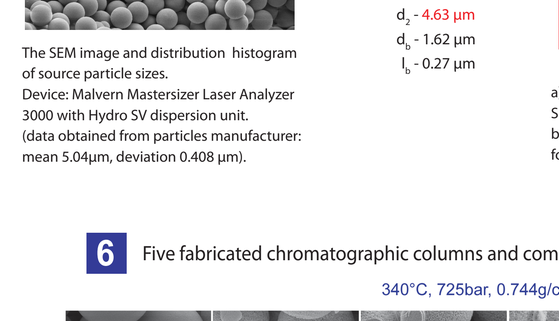
Phase 2 - Supercritical H_2O – etching and bridging
capillary packed with 5µm spheres + H_2O (p > 220 bar, T > 300°C)

Phase 3 - micro-HPLC analysis
structured 3D "hybrid" column (without frits) + chemical modification (C18)



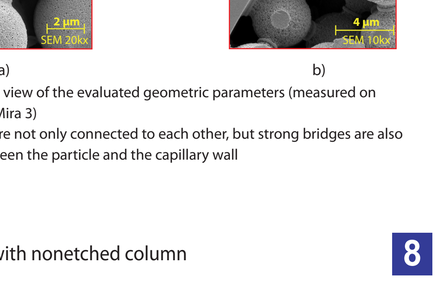
1 - CO_2 cylinder, 2 - high pressure pump, 3 - pressure sensor, 4 - on-off valve, 5 - filling reservoir, 6 - packed FS capillary, 7 - restrictor, 8 - H_2O reservoir, 9 - oxymeter, 10 - degasser, 11 - flowmeter, 12 - programmable moving device, 13 - heater (SCW generator), 14 - high pressure coupling, 15 - waste, 16 - process control and data collection, 17 - pump control, 18 - UV/VIS detector, 19 - computer controlled injection valve, 20 - splitter, 21 - "hybrid" column, 22 - data collection

Bridging Principle



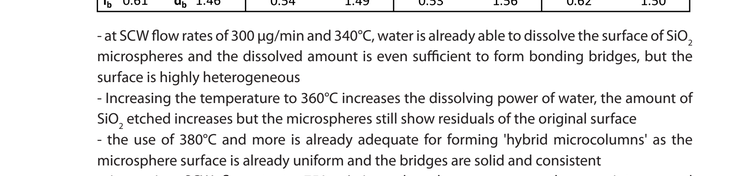
Wetting of differently spaced particles at low and high liquid content under the condition of mutual insolubility. The size and shape of the liquid bridge is determined by the distance between the particles and the thickness of the liquid layer.

A – in contact B – bridge forming distance C – too far for forming bridge



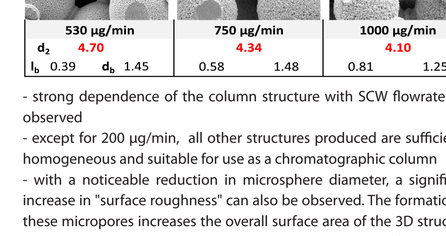
Formation of a bridge from the material removed from particles when the particle material dissolves. Assumption of the process : SCW is applied to the surface of the silica beads, a specific amount of SiO_2 dissolves, forms a silicic acid gel layer and reduces the diameter of the solid core of the beads from d_1 to d_2. This, in turn, creates a space between the surfaces of the beads $L_{SURFACE}>0$, which forms a liquid bridge subsequently while the distance between the centers L_{CENTRE} of the spheres remains constant.

SEM images of the connecting bridges produced under different conditions with visibly different centers



The rings in the middle of the bridges indicate a more complex process than was assumed on the left, the rings should be homogenous. The center white rings are the points of initial contact of solid particles where a strong connection is formed (a) and this structure is then further etched away as a whole by the SCW (b).

Scheme of the heater and continuous sintering process

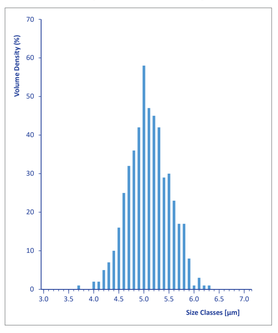


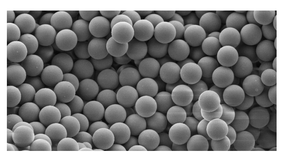
I. water preheating II. etching of the formed 3D structure III. liquid to solid bridge transformation IV. Liquid bridge formation V. microspheres preheating

The regions I-V are schematically shown parts where the different steps of the sintering process occur at the steady state. The shape of the regions, their size, location and perimeters cannot be determined in any simple way, also because these parameters change with the flow rate, the capillary movement speed and the temperature.

Results and Discussion

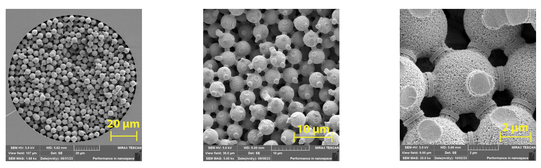
1 Original non-porous silica particles analysis





The SEM image and distribution histogram of source particle sizes.
Device: Malvern Mastersizer Laser Analyzer 3000 with Hydro SV dispersion unit.
(data obtained from particles manufacturer: mean 5.04µm, deviation 0.408 µm).

2 Example of randomly selected images showing fabricated column in overall view, at medium and high magnification



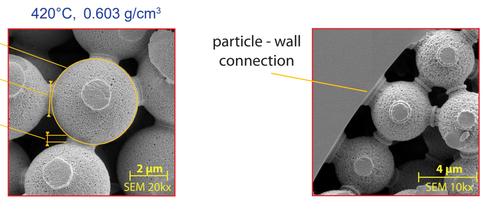
1700x 5000x 20000x

3 Evaluation parameters and detail of particle-wall connection

420°C, 0.603 g/cm³

d_2 - particle diameter
d_b - bridge diameter
l_b - bridge length

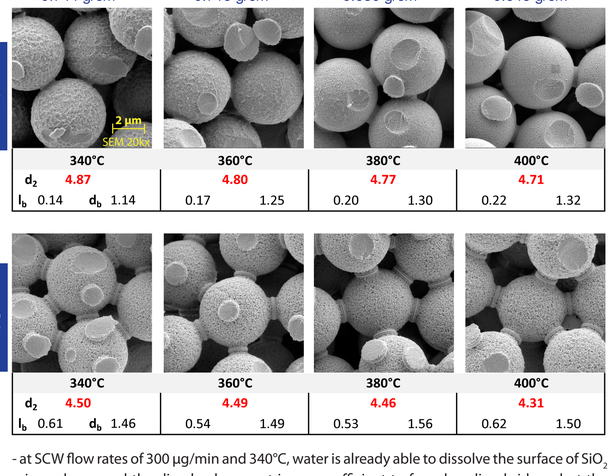
d_2 - 4.63 µm
d_b - 1.62 µm
l_b - 0.27 µm



a) b)

a) schematic view of the evaluated geometric parameters (measured on SEM Tescan Mira 3)
b) particles are not only connected to each other, but strong bridges are also formed between the particle and the capillary wall

4 Column structures made at 725 bar and different temperatures

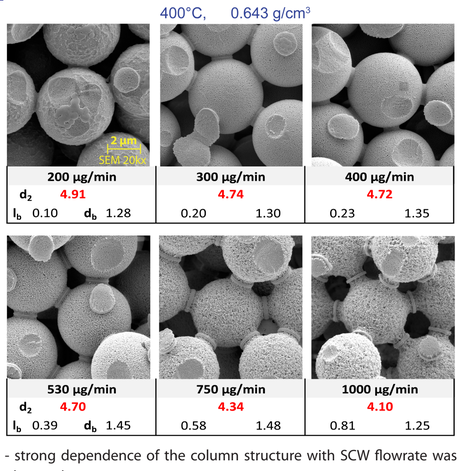


300 µg/min	340°C	360°C	380°C	400°C
d_2	4.87	4.80	4.77	4.71
l_b	0.14	0.17	0.20	0.22
d_b	1.14	1.25	1.30	1.32

750 µg/min	340°C	360°C	380°C	400°C
d_2	4.50	4.49	4.46	4.31
l_b	0.61	0.54	0.53	0.62
d_b	1.46	1.49	1.56	1.50

- at SCW flow rates of 300 µg/min and 340°C, water is already able to dissolve the surface of SiO_2 microspheres and the dissolved amount is even sufficient to form bonding bridges, but the surface is highly heterogeneous
- Increasing the temperature to 360°C increases the dissolving power of water, the amount of SiO_2 etched increases but the microspheres still show residuals of the original surface
- the use of 380°C and more is already adequate for forming 'hybrid microcolumns' as the microsphere surface is already uniform and the bridges are solid and consistent
- increasing SCW flowrate to 750µg/min makes the process much more intense and homogeneous columns can be prepared already at lower temperatures (340°C).

5 Effect of SCW flow rate change on column structures



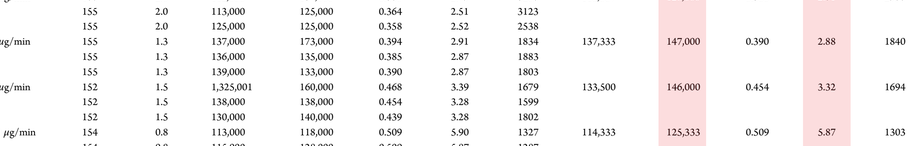
200 µg/min	300 µg/min	400 µg/min
d_2 4.91	4.74	4.72
l_b 0.10	0.20	0.23
d_b 1.28	1.30	1.35

530 µg/min	750 µg/min	1000 µg/min
d_2 4.70	4.34	4.10
l_b 0.39	0.58	0.81
d_b 1.45	1.48	1.25

- strong dependence of the column structure with SCW flowrate was observed
- except for 200 µg/min, all other structures produced are sufficiently homogeneous and suitable for use as a chromatographic column
- with a noticeable reduction in microsphere diameter, a significant increase in "surface roughness" can also be observed. The formation of these micropores increases the overall surface area of the 3D structure and can play a positive role in both chromatographic and, for example, catalytic processes.

6 Five fabricated chromatographic columns and comparison with nonetched column

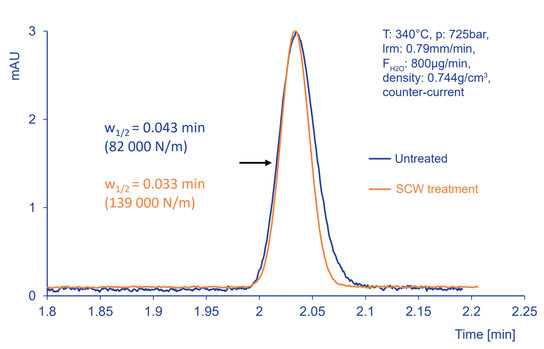
340°C, 725bar, 0.744g/cm³



non etched	400 µg/min	600 µg/min	800 µg/min	1000 µg/min	1200 µg/min
d_2 5.01	4.69	4.63	4.44	4.28	3.95
l_b 0	0.22	0.37	0.48	0.69	0.80
d_b 0	1.15	1.42	1.39	1.50	1.30

counter-current mode, 100 µm i.d. fused silica capillary, capillary movement rate=0.79mm/min, P=725 bar, SCW flowrate=400-1200µg/min, SCW temperature=340°C, SCW density=0.744 g/cm³.

7 Peak profiles of unretained compound (toluene), eluted from unmodified chromatographic column



- Because of different total porosities of the columns (0.313 for the untreated one and 0.454 for the SCW-treated one), the amount of mobile phase in each column was different, and the difference resulted in different final flow rates (246 and 328 nL/min, respectively) to obtain the same retention times. We observed a change of efficiency from 82 000 to 139 000 plates/m when measured by width of the peak at half of its height. When efficiencies were calculated from statistical moments (by DataApex Clarity 5.02 software), values 98 000 for untreated column and 160 000 for SCW-treated column were obtained.

- from Table 1, it can be seen that permeability k of particles after treatment grows linearly with SCW flow rate. When compared with non-etched particles, we can observe more than 6× increase of column permeability. From chromatographic point of view, higher permeability of the column is preferred because it reduces the pressure needed in the system. Also, a more viscous mobile phase or high flowrate can be used for separation.

Table 1. Measured Chromatographic Parameters (Left Side), Average Values n = 3 (Right Side)

SCW flow	length [mm]	dp [MPa]	N/m $W_{50\%}$[a]	N/m stat.mom.[b]	ε total	k [× 10^{-14}m²]	E	N/m $W_{50\%}$[a]	N/m stat.mom.[b]	ε total	k [× 10^{-14}m²]	E
nonetched	153	3.1	96,000	96,000	0.313	1.13	9581	86,667	97,333	0.313	1.13	11931
	153	3.1	82,000	98,000	0.312	1.13	13,132					
	153	3.1	82,000	98,000	0.315	1.14	13,080					
400 µg/min	155	2.0	112,000	134,000	0.358	2.49	3205	116,667	128,000	0.360	2.51	2955
	155	2.0	113,000	125,000	0.364	2.51	3123					
	155	2.0	125,000	125,000	0.358	2.52	2538					
600 µg/min	155	1.3	137,000	173,000	0.394	2.91	1834	137,333	147,000	0.390	2.88	1840
	155	1.3	136,000	135,000	0.385	2.87	1883					
	155	1.3	139,000	133,000	0.390	2.87	1803					
800 µg/min	152	1.5	1,325,001	160,000	0.468	3.39	1679	133,500	146,000	0.454	3.32	1694
	152	1.5	138,000	138,000	0.454	3.28	1599					
	152	1.5	130,000	140,000	0.439	3.28	1802					
1000 µg/min	154	0.8	113,000	118,000	0.509	5.90	1327	114,333	125,333	0.509	5.87	1303
	154	0.8	115,000	128,000	0.509	5.87	1287					
	154	0.8	115,000	130,000	0.508	5.84	1294					
1200 µg/min	145	1.0	117,000	156,000	0.563	6.76	1080	121,333	141,333	0.563	6.88	990
	145	1.0	123,000	125,000	0.562	6.94	953					
	145	1.0	124,000	143,000	0.564	6.94	937					

[a]Calculated assuming Gaussian concentration profile. [b]Calculated from statistical moments by Data Apex clarity 5.02 software.

8 Chromatographic evaluation of SCW treated and untreated ODS modified columns

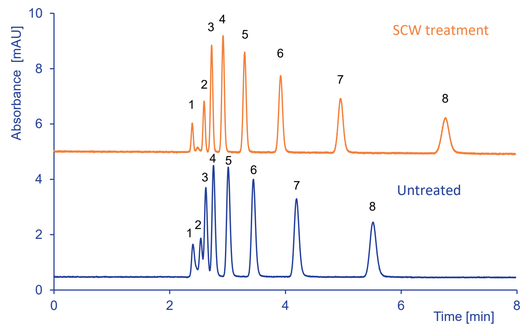


Table S1: Chromatographic separation of alkylbenzenes on SCW-treated column and ODS-modified column

Peak	Reten. time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Compound Name
1	2.390	0.040	0.909	0.00	19833	130483		Uracil
2	2.593	0.043	1.182	0.08	19893	130874	2.879	Benzene
3	2.722	0.050	1.167	0.14	16475	108390	1.644	Toluene
4	2.922	0.053	1.000	0.22	16682	109753	2.284	Ethylbenzene
5	3.295	0.063	1.059	0.38	15041	98953	3.776	Propylbenzene
6	3.914	0.077	1.150	0.64	14483	95285	5.226	Butylbenzene
7	4.952	0.100	1.071	1.07	13629	89666	6.946	Pentylbenzene
8	6.766	0.146	1.075	1.83	11827	77810	8.691	Hexylbenzene

Column: i.d. = 0.1 mm, length = 152 mm, stationary phase: C18, mobile phase: 50% acetonitrile / water

Table S2: Chromatographic separation of alkylbenzenes on SCW-untreated column and ODS-modified column

Peak	Reten. time [min]	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]	Compound Name
1	2.400	0.057	1.385	0.00	9937	62109		Uracil
2	2.537	0.053	0.917	0.06	12533	78328	1.466	Benzene
3	2.623	0.057	1.067	0.09	11873	74206	0.930	Toluene
4	2.757	0.063	1.200	0.15	10496	65598	1.311	Ethylbenzene
5	3.010	0.070	1.294	0.25	10243	64022	2.242	Propylbenzene
6	3.447	0.080	1.200	0.44	10283	64270	3.485	Butylbenzene
7	4.190	0.097	1.250	0.75	10408	65053	4.965	Pentylbenzene
8	5.517	0.127	1.088	1.30	10508	65678	7.010	Hexylbenzene

Column: i.d. = 0.1 mm, length = 160 mm, stationary phase: C18, mobile phase: 50% acetonitrile / water

Measurements with non retained substance in the system (toluene) gave only indicative information about the effect of SCW treatment on efficiency; resulting efficiency mainly reflects the homogeneity of the column bed. In the next step, one of the SCW-treated columns (800 µg/min) and one column packed with untreated particles were submitted to ODS modifications.
For SCW treated column, the performance for uracil (as a practically unretained analyte) and benzene (as a compound with low retention) confirmed values (130 000 plates/m), which we obtained for toluene (in 90% ACN). By comparing Tables S1 and S2, it is possible to determine the effect of treatment on the spectrum of analytes with higher retention factors.

9 Test of monolith fabrication reproducibility, statistics data

Table S4: Test of monolith fabrication reproducibility, statistics data

Column No._cut	spheres diameter d_2 [µm]	bridge length l_b [µm]	bridge diameter d_b [µm]	Column No.		spheres diameter d_2 [µm]	bridge length l_b [µm]	bridge diameter d_b [µm]
I_1	4.660	1.390	0.310	I	Average [µm]	4.660	1.428	0.335
I_2	4.680	1.380	0.330		SD [µm]	0.043	0.052	0.031
I_3	4.700	1.490	0.380		RSD [%]	0.927	3.634	9.281
I_4	4.600	1.450	0.320					
II_1	4.700	1.480	0.330	II	Average [µm]	4.700	1.488	0.319
II_2	4.760	1.490	0.310		SD [µm]	0.043	0.017	0.009
II_3	4.680	1.470	0.315		RSD [%]	0.920	1.148	2.679
II_4	4.660	1.510	0.320					
III_1	4.660	1.500	0.340	III	Average [µm]	4.710	1.495	0.340
III_2	4.700	1.490	0.360		SD [µm]	0.039	0.013	0.022
III_3	4.740	1.510	0.310		RSD [%]	0.813	0.864	6.354
III_4	4.740	1.480	0.350					
IV_1	4.660	1.490	0.340	IV	Average [µm]	4.700	1.501	0.314
IV_2	4.756	1.512	0.307		SD [µm]	0.042	0.009	0.018
IV_3	4.686	1.500	0.302		RSD [%]	0.891	0.600	5.620
IV_4	4.680	1.500	0.306					
V_1	4.760	1.480	0.360	V	Average [µm]	4.700	1.497	0.337
V_2	4.660	1.490	0.320		SD [µm]	0.053	0.021	0.021
V_3	4.680	1.520	0.330		RSD [%]	1.126	1.391	6.183
					Average [µm]	**4.693**	**1.481**	**0.329**
				column to column	SD [µm]	**0.019**	**0.031**	**0.012**
					RSD [%]	**0.410**	**2.060**	**3.577**

The column preparation reproducibility investigations have included a (single) column homogeneity test and a column-to-column reproducibility test. Table S4 includes the "aggregated" column-to-column statistics indicating very good column-to-column reproducibility of the monolith fabrication via the SCW-induced etching/bridging procedure.

Conclusion

This pilot work presents a new and unique method for preparing 3D homogeneous structures using supercritical water's ability to dissolve silica. The method's high variability is due to the wide range of applicable temperatures and pressures that can be used to tune the properties of supercritical water. Chromatographic columns prepared using this method exhibit high separation efficiency while maintaining high permeability. The microspheres are not only connected to each other but also to the capillary wall, resulting in a rigid cartridge that is free from any movement. Therefore, unlike conventional packed columns, this column does not require frits at its ends, it can be freely shortened at will if necessary, and the mobile phase can flow through the column in either direction. As regards the mechanical stability and durability of the 3D framework, we did not observe any decrease of column efficiency (even after several drying/moistening cycles), which itself is a good indicator of 3D structure mechanical stability. Depending on the conditions, there is a significant reduction in particle diameter from 5.04 to 3.95 µm, resulting in a decrease in hydrodynamic resistance. This effect would be more pronounced for smaller original microspheres (3.0 or 1.7 µm). The use of pure water throughout the process and the absence of heteroatoms on the surface of the structure can be crucial in biochemical analyses. In order to assess the performance of the new type of monolithic columns, an SCW-treated column and a discrete microsphere-packed column were both chemically modified to introduce C18 stationary phase. A simple comparison using a mixture of alkylbenzenes indicated superior performance of the SCW-treated, bridged-microsphere column over the standard, discrete microsphere-packed column.

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