Electronic Supplementary Material (ESI) for Analytical Methods.
This journal is © The Royal Society of Chemistry 2019

Material and methods

As reported recently[11], programming a saw tooth gradient over a range from 0 to 100 % desorption promoting solvent was possible without any limitations with the chromatographic data system Chromeleon (Thermo Fisher Scientific, version 7.2), in contrast to other investigated software packages limited in the number of possible entries in the gradient time table. Furthermore, comparing the driver configuration between Agilent and Thermo Fisher Scientific HPLC pumps, gradient programming without limited number of entries in the gradient time table was only possible for pumps from Thermo Fisher Scientific.

Mobile phase components and polymer standards

All used solvents were HPLC grade. Acetone, acetonitrile, methanol, non-stabilized tetrahydrofuran (THF), and toluene were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. Poly(dimethylsiloxane) (PDMS) standards with viscosities of 350 mPa·s and 1000 mPa·s were obtained from Wacker Chemie AG (Burghausen, Germany). The used poly(methyl methacrylate) (PMMA), poly(vinyl chloride) (PVC) and poly(propylene glycol) (PPG) were purchased from Agilent (Church Stretton, UK). All used polymer standards were dissolved in THF.

Optimization of LC flow rate

The effect of various LC flow rates was measured with a Thermo Fisher Scientific (Waltham, USA) Ultimate 3000 HPLC system equipped with a binary pump. For detection, an Agilent (Waldbronn, Germany) 385 ELSD modified with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. (Mississauga, Ontario, Canada) at 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.6 SLM (standard liter per minute) gas flow was used[18]. The

measurements were done with an Agilent Poroshell HILIC (50x4.6 mm, 2.7 µm) and a silicone oil of 1000 mPa·s (Wacker Chemie AG, Burghausen, Germany). The saw tooth gradient was programmed with an effective step height of 0.2 % and a height of the negative backward gradient step of 6.0 % (compare[11]) and LC flow rates of one, two, and three $mL \cdot min^{-1}$.

Evaluation measurements of various saw tooth gradient profiles

For comparison of three different discussed saw tooth profiles, a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump, the above-mentioned modified Agilent 385 ELSD and a Thermo Fisher Scientific Accucore C18 (50x4.6 mm, 2.6 µm) were used. In each case the saw tooth gradients were performed with an effective step height of 1.0 % and an effective step length of 0.2 min. The height of the negative backward gradient step was 6.0 % or the maximally possible value of 100 % and for the multicomponent saw tooth gradient in each step during the height of the negative backward gradient step the mobile phase composition was changed to water instead of methanol followed by a reconditioning step back to a mixture of THF and methanol before switching to the positive slope. A PMMA standard with an average molecular weight of 107,000 $g \cdot mol^{-1}$ at a concentration of 20 $mg \cdot mL^{-1}$ was used as analyte.

The setup of the ternary saw tooth gradient setup separating PMMA (19,700 $g \cdot mol^{-1}$), polypropylene glycol (PPG, 18,000 $g \cdot mol^{-1}$), and PDMS (18,600 $g \cdot mol^{-1}$) at a concentration of 20 $mg \cdot mL^{-1}$ respectively, consisted of two different saw tooth gradient approaches: Starting at 100 % methanol (adsorption promoting solvent) a first saw tooth gradient with an effective step length of 0.6 min, an effective step height of 1.0 % and a height of the negative backward gradient step of 6.0 % were performed with acetone as desorption promoting solvent (elution of PMMA und PPG). Subsequently, at 100 % acetone the same saw tooth gradient was used but with THF as a stronger desorption promoting solvent (elution of PDMS) while acetone acted as an adsorption promoting solvent.

The optimization of the saw tooth gradient with design of experiments (DoE) by Taguchi was done on a Thermo Fisher Scientific (Waltham, USA) Vanquish UHPLC with UV detection at 215 nm. The investigated parameters of V11 for the optimization of the saw tooth gradient profile by DoE are 9 % for the height of the negative backward gradient step, 1.0 mL for the lower plateau, and 0.8 % effective step height, and were performed from 0 % non-stabilized THF (100 % methanol) to 100 % THF (0 % methanol).

Comparison of different LC columns

Various types of liquid chromatographic stationary phases were compared in terms of separating PMMA, PDMS, PVC, and PPG at a concentration of 20 $mg \cdot mL^{-1}$ respectively. SIS-Table 1 gives an overview of the specific data. The measurements were performed on a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump and a modified Agilent 385 ELSD. Each polymer was separately measured on each column with a linear gradient starting from 100 % methanol and finishing at 100 % THF.

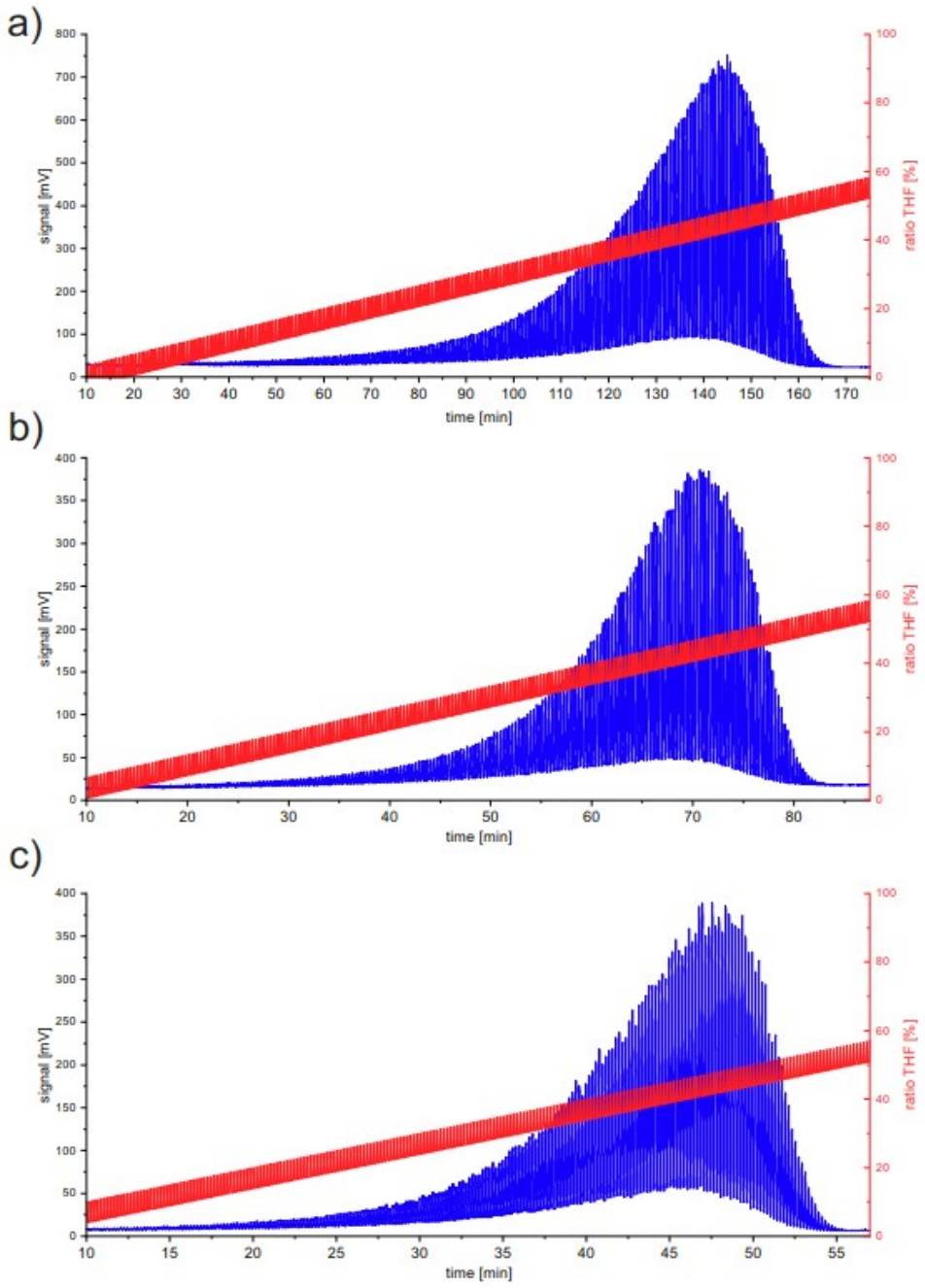
SIS-Table 1. Overview of used LC columns separating PMMA, PVC, PDMS, and PPG with an average molecular mass of approximately 20 kDa, the categories are an indicator for elution order of the different polymers: category 1 - PMMA-PPG-PDMS-PVC, category 2 - PMMA-PPG-PVC-PDMS, category 3 - PPG-PMMA-PDMS-PVC, category 4 - PDMS-PMMA-PPG (PVC irreversibly remained at the column), and category 5 - PPG-PDMS-PMMA-PVC.

Category	Manufacturer	Name	Dimensions, particle type	Evaluation
1	ZirChrom	Diamond Bond C18	150x4.6 mm, 5 µm, fully porous	zircon based, carbon coated, extended pH range
1	Phenomenex	Luna Omega PS C18	150x4.6 mm, 5 µm, fully porous, 100 Å	mixed mode C18 with positive charge for additional ionic interactions
1	Tosoh	TSK Gel Boronate 5PW	75x7.5 mm, 10 µm, polymer	polymer based, m-aminophenylboronate coated, for affinity chromatography
2	Merck	Chromolith Performance RP C18	100x4.6 mm, monolithic column	monolithic column

2	Microsolv Technology	Cogent Bidentate C18	150x4.6 mm, 4.2 µm, fully porous, 100 Å	no silanol activity, C18 chain directly to Silica-hydrid surface bonded
3	Agilent	PLRP-S	50x4.6 mm, 10 µm, polymer, 4000 Å	polymer based, for biomolecule separation
3	Thermo-Fisher	Omnipac Pax-500	250x4.6 mm, 8.5 µm, polymer	mixed mode polymer phase with anion exchange interactions
3	Thermo-Fisher	Accucore C18	50x4.6 mm, 2.6 µm, core shell, 80 Å	typical C18 column, non-polar interactions
3	Phenomenex	Kinetex F5	50x4.6 mm, 2.6 µm, core shell, 100 Å	pentafluorophenyl column for steric separations
3	Phenomenex	Kinetex Biphenyl	50x4.6 mm, 2.6 µm, core shell, 100 Å	hydrophobic, aromatic and polar interactions
3	Phenomenex	Luna Phenylhexyl	50x4.6 mm, 5 µm, fully porous	hydrophobic, aromatic interactions
4	Thermo-Fisher	Hypercarb	100x4.6 mm, porous graphitic carbon	graphitic carbon
5	Agilent	PL gel Mixed C	300x7.5 mm, 5 µm, polymer	SEC column, molar mass range 200 to 2,000,000 $g \cdot mol^{-1}$

HRP-HPLC using a SEC column

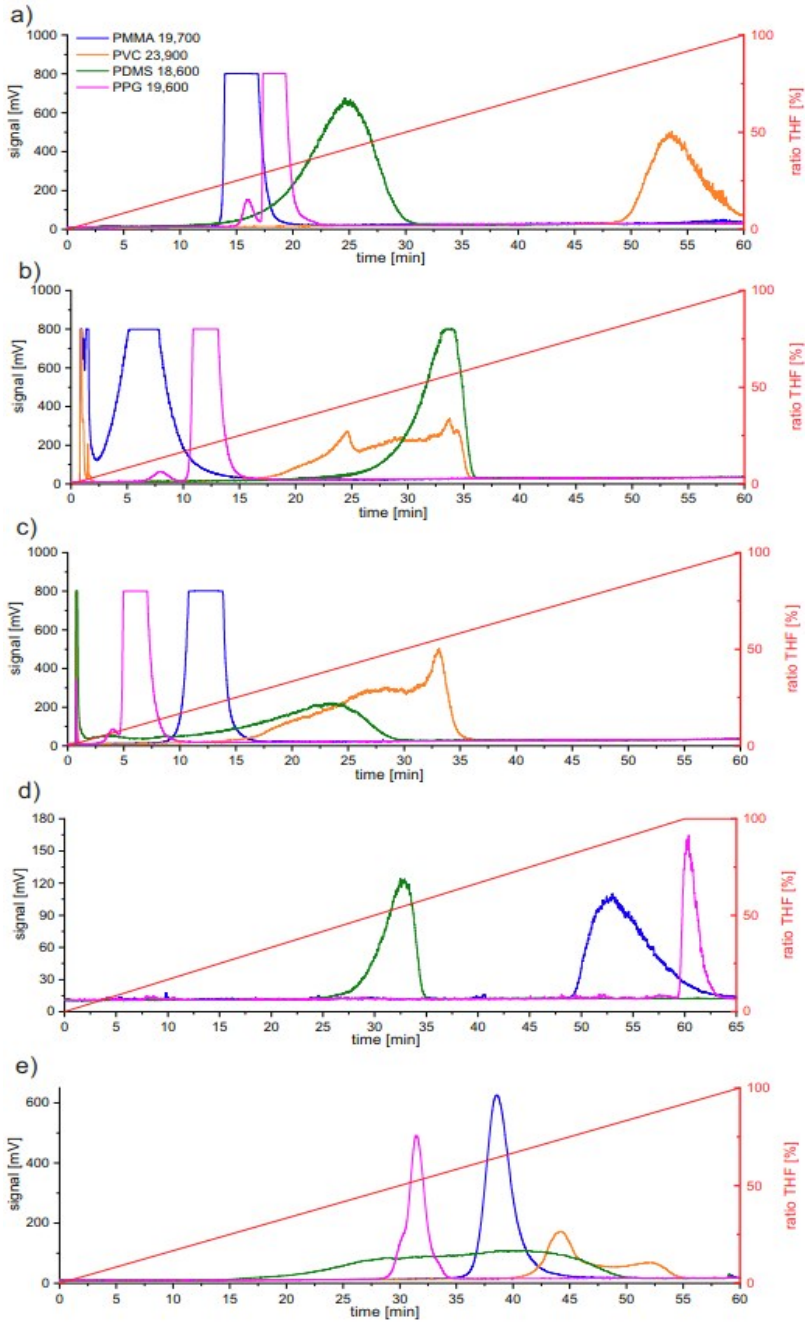
SEC measurements were performed with a Polymer Standards Service (Mainz, Germany) SECcurity GPC 1200 system equipped with a refractive index detector. Data evaluation was done with the SEC software package WinGPC UniChrom 8.2. For polymer HPLC, a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump and the modified Agilent 385 ELSD was used. An Agilent PL gel Mixed C column (300x7.5 mm, 5 µm) was used for the separation of PMMA standards with an average molecular weight of 19,700 $g \cdot mol^{-1}$, 107,700 $g \cdot mol^{-1}$, 690,000 $g \cdot mol^{-1}$, and 1,600,000 $g \cdot mol^{-1}$ with THF as eluent in SEC mode and acetonitrile as adsorption promoting solvent and THF as desorption promoting solvent in polymer HPLC mode.



SIS-Figure 1. HRP-HPLC measurement of PMMA with average molecular weight of 107,000 g·mol^{-1} on a Poroshell HILIC (50x4.6 mm, 2.7 µm) using methanol as adsorption promoting solvent and THF as desorption promoting solvent and a saw tooth gradient with an effective step height of 0.2 % and a height of the negative backward gradient step of 6.0 %; the figure depicts the effect of varying flow rate of 1 mL·min^{-1} (a), 2 mL·min^{-1} (b), and 3 mL ·min^{-1} (c) on the total runtime and peak separation.

SIS-Table 2. Calculated values for peak height difference between the integration modes of perpendicular line dropping and valley-to-valley setting; the DoE number refers to the DoE described in[11].

Number in DoE	height of the negative backward gradient step [%]	Peak height difference [mAU]
1	3.0	624.2
2	3.0	1090.0
3	3.0	1226.0
4	3.0	873.4
5	6.0	0.2
6	6.0	277.6
7	6.0	335.2
8	6.0	578.9
9	9.0	0.2
10	9.0	135.8
11	9.0	8.6
12	9.0	14.9
13	12.0	0.2
14	12.0	0.2
15	12.0	0.2
16	12.0	0.4



SIS-Figure 2. Separation of PMMA (blue line), PVC (orange line), PDMS (green line), and PPG (pink line) with an average molecular mass of approximately 20 kDa and a concentration of 20 mg·mL^{-1} respectively, methanol as adsorption promoting solvent, THF as desorption promoting solvents (linear gradient) at a Diamond Bond C18 (150x4.6 mm, 5 µm) (a), a Chromolith RP C18 (100x4.6 mm, monolithic) (b), a PLRP-S (50x4.6 mm, 10 µm, polymer material) (c), a Hypercarb (100x4.6 mm, porous graphitic carbon) (d), and a PLgel Mixed C (300x7.5 mm, 5 µm, SEC column) (e).

SIS-Table 3. SEC results of the preparative HRP-HPLC separation of PDMS with viscosity of 350 mPa·s, the values for the number average molar mass M_n, weight average molar mass M_w, and average molar mass at peak maximum M_p were measured with an RI detector and calculated with the software WinGPC Unichrom 8.2; the fraction number refers to those of Figure 6.

	weight of fraction [mg]	M_n [g·mol^{-1}]	M_w [g·mol^{-1}]	M_p [g·mol^{-1}]	Polydispersity
original sample	----	9460	19000	18600	2.01
FC 1	0.1	2390	2580	2880	1.08
FC 2	0.1	2460	2660	2990	1.08
FC 3	0.2	2630	2770	3060	1.05
FC 4	0.3	2690	2850	3150	1.06
FC 5	0.3	2690	2900	3210	1.08
FC 6	0.5	2780	2980	3310	1.07
FC 7	0.5	2790	3030	3380	1.09
FC 8	0.1	2910	3120	3450	1.07
FC 9	0.7	3050	3260	3570	1.07
FC 10	0.1	3110	3340	3670	1.07
FC 11	0.5	3290	3510	3850	1.07
FC 12	0.0	3420	3630	3990	1.06
FC 13	1.0	3540	3760	4130	1.06
FC 14	0.9	3670	3910	4300	1.07
FC 15	1.1	3840	4070	4410	1.06
FC 16	1.2	3970	4230	4630	1.07
FC 17	1.2	4170	4420	4820	1.06
FC 18	1.7	4360	4620	5030	1.06
FC 19	1.5	4550	4820	5220	1.06
FC 20	1.5	4690	5040	5500	1.07
FC 21	1.7	5020	5320	5780	1.06
FC 22	2.0	5210	5580	6040	1.07
FC 23	2.0	5550	5900	6450	1.06
FC 24	2.8	5860	6220	6810	1.06
FC 25	2.6	6250	6660	7230	1.07
FC 26	1.8	6620	7050	7740	1.06
FC 27	3.4	7090	7560	8220	1.07
FC 28	3.8	7530	8050	8870	1.07
FC 29	4.3	8040	8610	9470	1.07
FC 30	5.3	8650	9280	10200	1.07
FC 31	5.8	9340	10000	11000	1.07
FC 32	6.3	10200	10900	12000	1.07
FC 33	10.1	11100	11900	13100	1.07
FC 34	8.7	12200	13100	14300	1.07
FC 35	8.0	13400	14400	15800	1.07
FC 36	11.6	15000	16000	17600	1.07
FC 37	13.2	16800	18000	19800	1.07
FC 38	14.8	19000	20500	22300	1.08
FC 39	21.7	21700	23500	25800	1.08
FC 40	19.5	25400	27600	30300	1.09
FC 41	17.7	30300	33200	36300	1.10
FC 42	21.8	38000	41300	44600	1.09
FC 43	20.4	48000	53000	57300	1.10
FC 44	15.2	60100	70200	76200	1.17