Supplementary material

Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra

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Table S1. Properties of SPE cartridges used for extraction.

Cartridge	Manufacturer	Material	Average particle size (µm)	Average pore size (Å)	Bed mass (mg)	Volume (mL)
Bond Elut PPL	Agilent	modified styrene-divinylbenzene copolymer	125	150	500	6
Oasis HLB	Waters	poly(N-vinyl-pyrrolidone-divinylbenzene)	30	80	500	6
Bakerbond Octadecyl C18	J. T. Baker	C18	47 – 60	60	500	6

Table S2. SPE extraction method.

Step	Solvent	Delivery rate	Soak time
Wash cartridge	4 mL methanol	10 mL min^{-1}	15 s
Wash cartridge	3 mL methanol	10 mL min^{-1}	20 s
Wash cartridge	4 mL ultrapure water	10 mL min^{-1}	10 s
Wash cartridge	3 mL 0.01 M HCl	10 mL min^{-1}	10 s
Wash cartridge	4 mL 0.01 M HCl	10 mL min^{-1}	10 s
Load	50 mL sample at 10 mL min^{-1}	5 mL min^{-1}	
Wash cartridge	3 mL 0.01 M HCl	10 mL min^{-1}	10 s
Wash cartridge	3 mL 0.01 M HCl	10 mL min^{-1}	10 s
Dry for 10 min with N_2			
Elute cartridge	2 mL methanol	5 mL min^{-1}	120 s
Elute cartridge	2 mL methanol	5 mL min^{-1}	120 s
Dry cartridge	6 mL air	5 mL min^{-1}	

Table S3. Additional properties of the model compounds.

Index	Name	Supplier	Purity (%)
1	D-Sorbitol	Sigma-Aldrich	98
2	D-Glucoronic acid	Alfa Aesar	98
3	Cellobiose	Merck	-
4	Naphthalene-1,5-disulfonic acid	Sigma-Aldrich	97
5	Gallic acid	Merck	-
6	Glutaric acid	Sigma-Aldrich	99
7	1,2-Dihydroxybenzene	Sigma-Aldrich	≥ 99
8	4-Hydroxybenzoic acid	Merck	98
9	Phthalic acid	Merck	99.5
10	Chlorogenic acid	Sigma-Aldrich	≥ 95
11	Vanillic acid	Sigma-Aldrich	≥ 97
12	(-)-Epicatechin	Fluka	> 90
13	2-Hydroxy-2-phenylacetic acid	Sigma-Aldrich	99
14	Syringic acid	Fluka	≥ 97
15	Naphthalene-2-sulfonic acid	Sigma-Aldrich	≥ 98
16	Ferulic acid	Fluka	≥ 98
17	12-Hydroxydodecanoic acid	Sigma-Aldrich	97
18	Sodium dodecyl sulfate	Merck	≥ 99

Table S4. Gradient used for UPLC-QTOF-MS of the model compounds.

Time (min)	Eluent A (%)	Eluent B (%)
0	99.5	0.5
2	99.5	0.5
7.21	0.1	99.9
10.21	0.1	99.9
10.31	99.5	0.5
10.5	99.5	0.5

Table S5. Extraction efficiencies of model compounds for the PPL and the HLB cartridge and the response factor (peak area/concentration) in negative ESI, * determined in positive ESI.

Index	Substance	Extraction efficiency (%)						Response
		PPL 1	PPL 2	Mean	HLB 1	HLB 2	Mean	(L µmol^{-1})
1	D-Sorbitol	0.0*	0.0*	0.0	0.2*	1.3*	0.8	1252 (7855)*
2	D-Glucuronic acid	0.0*	0.0*	0.0	0.0*	0.0*	0.0	7424 (538)*
3	Cellobiose	0.0*	0.0*	0.0	0.0*	3.1*	1.5	417 (47523)*
4	Napthalene-1,5-disulfonic acid	1.6	2.1	1.9	3.3	2.8	3.1	36269
5	Gallic acid	2.3	2.4	2.3	83.6	88.5	86.0	38546
6	Glutaric acid	46.6	44.9	45.8	76.8	75.5	76.2	7310
7	1,2-Dihydroxy-benzene	64.2	64.2	64.2	77.4	79.8	78.6	5006
8	4-Hydroxy-benzoic acid	93.0	92.7	92.8	76.2	76.0	76.1	20896
9	Phthalic acid	95.2	90.2	92.7	86.5	75.8	81.2	59725
10	Chlorogenic acid	91.5	94.4	93.0	82.2	83.3	82.7	154810
11	Vanillic acid	97.6	86.7	92.2	68.3	74.4	71.3	3572
12	(-)-Epicatechin	71.3	71.9	71.6	32.8	34.7	33.8	58996
13	2-Hydroxy-2-phenylacetic acid	93.6	93.3	93.5	77.2	77.4	77.3	26152
14	Syringic acid	98.3	92.5	95.4	74.1	68.5	71.3	8471
15	Napthalene-2-sulfonic acid	78.9	81.6	80.3	0.0	0.0	0.0	271306
16	Ferulic acid	95.3	95.4	95.4	73.4	77.3	75.4	41299
17	12-Hydroxy dodecanoic acid	72.3	66.7	69.5	63.8	65.2	64.5	331970
18	Sodium dodecyl sulfate	59.0	66.7	62.9	0.0	1.1	0.5	1674462

Table S6. DOC concentrations of SEC fractions B – D of the DOM of the freshwater sample I and the extracts and permeates of the three SPE cartridges, * % of original sample, LOD = 10 µg L^{-1}, LOQ = 40 µg L^{-1}, n.d. = not determined, $\bar{x}$ = arithmetical mean, the mean molecular weight M_n was only determined for fraction B ('humic substances') and no fraction A was determined in these samples.

	Sum of B to D		**Fraction B**			**Fraction C**		**Fraction D**	
Sample I	**µg L^{-1} C**	**%***	**µg L^{-1} C**	**%***	**M_n (g mol^{-1})**	**µg L^{-1} C**	**%***	**µg L^{-1} C**	**%***
original I	9424		8626		1012	572		226	
original II	9506		8676		1032	606		224	
$\bar{x}$ original	9465		8651			589		225	
PPL extract I	6672	70	5858	68	1000	588	100	226	100
PPL extract II	5774	61	5004	58	1026	578	98	192	85
$\bar{x}$ extracts	6223	66	5431	63		583	99	209	93
PPL permeate I	612	6	500	6	837	78	13	< 40	n.d.
PPL permeate II	484	5	366	4	744	80	14	< 40	n.d.
$\bar{x}$ permeate	548	6	433	5		79	13	< 40	n.d.
∑ extracts + permeates	6771	72	5864	68		662	112	> 209	> 93
HLB extract I	2200	23	1356	16	755	420	71	150	67
HLB extract II	1846	20	1064	12	742	344	58	134	60
$\bar{x}$ extracts	2023	21	1210	14		382	65	142	63
HLB permeate I	292	3	214	2	745	78	13	< 10	n.d.
HLB permeate II	290	3	230	3	695	60	10	< 10	n.d.
$\bar{x}$ permeates	291	3	222	3		69	12	< 10	n.d.
∑ extracts + permeates	2314	24	1432	17		451	77	142	63
C18 extract I	6748	71	5716	66	1106	834	142	198	88
C18 extract II	5394	54	4584	53	1092	644	109	166	74
$\bar{x}$ extracts	6071	67	5150	60		739	125	182	81
C18 permeate I	1294	14	1122	13	781	114	19	58	26
C18 permeate II	1264	13	1066	12	789	150	25	48	21
$\bar{x}$ permeates	1279	14	1094	13		132	22	53	24
∑ extracts +permeates	7350	78	6244	72		871	148	235	104

Table S7. DOC concentrations of SEC fractions A – D of the DOM of the freshwater sample II and the extracts and permeates of the three SPE cartridges from sample II, * % of original sample, LOD = 10 µg L^{-1}, LOQ = 40 µg L^{-1}, n.d. = not determined, the mean molecular weight M_n was only determined for fraction B ('humic substances').

	Sum of A to D		**Fraction A**		**Fraction B**			**Fraction C**		**Fraction D**	
Sample II	**µg L^{-1} C**	**%***	**µg L^{-1} C**	**%***	**µg L^{-1} C**	**%***	**M_n (g mol^{-1})**	**µg L^{-1} C**	**%***	**µg L^{-1} C**	**%***
original	4371		324		3150		783	571		326	
PPL extract	2678	61	55	17	1763	56	777	548	96	312	96
PPL permeate	383	9	< 10	n.d.	304	10	n.d.	< 40	n.d.	64	20
∑	3061	70	> 55	> 17	2067	66		> 548	> 96	376	116
HLB extract	1661	38	68	21	921	29	657	419	73	253	78
HLB permeate	366	8	< 40	n.d.	217	7	686	76	13	44	13
∑	2027	46	> 68	> 21	1138	36		495	86	297	91
C18 extract	2160	49	58	18	1516	48	734	313	55	273	84
C18 permeate	1711	39	70	22	846	27	776	574	101	221	68
∑	3871	88	128	40	2362	75		887	156	494	152

Table S8. DOC concentrations of SEC-OCD fractions A –D of the extracts for freshwater sample II using additional 4 ml of methanol (MeOH 2) and 4 ml of acetonitrile (ACN) for elution. LOD = 10 µg L^{-1}, LOQ = 40 µg L^{-1}.

Sample	**Sum of A to D**		**Fraction A**	**Fraction B**	**Fraction C**	**Fraction D**
	µg L^{-1} C	**% of original**	**µg L^{-1} C**	**µg L^{-1} C**	**µg L^{-1} C**	**µg L^{-1} C**
original	4371		324	3150	571	326
PPL MeOH 2	90	2	< 10	59	< 40	< 40
PPL ACN	76	2	< 40	< 40	< 40	< 40
HLB MeOH 2	325	7	< 10	197	95	< 40
HLB ACN	116	3	< 10	< 40	< 40	< 10
C18 MeOH 2	52	1	< 10	< 40	< 10	< 40
C18 ACN	42	1	< 10	< 10	< 40	< 10

Table S9. Nitrogen containing compounds in the original samples I and III and in the extracts.

	Sample I				**Sample III**			
	Original	**PPL**	**HLB**	**C18**	**Original**	**PPL**	**HLB**	**C18**
Sum of relative intensities (%)	4.7	2.9	3.7	1.4	4.2	3.9	4.1	3.0
Proportion of the original (%)		61	79	30		93	98	71
Number of sum formulae	405	247	301	96	216	182	176	148
Proportion of the original (%)		61	74	24		84	81	69

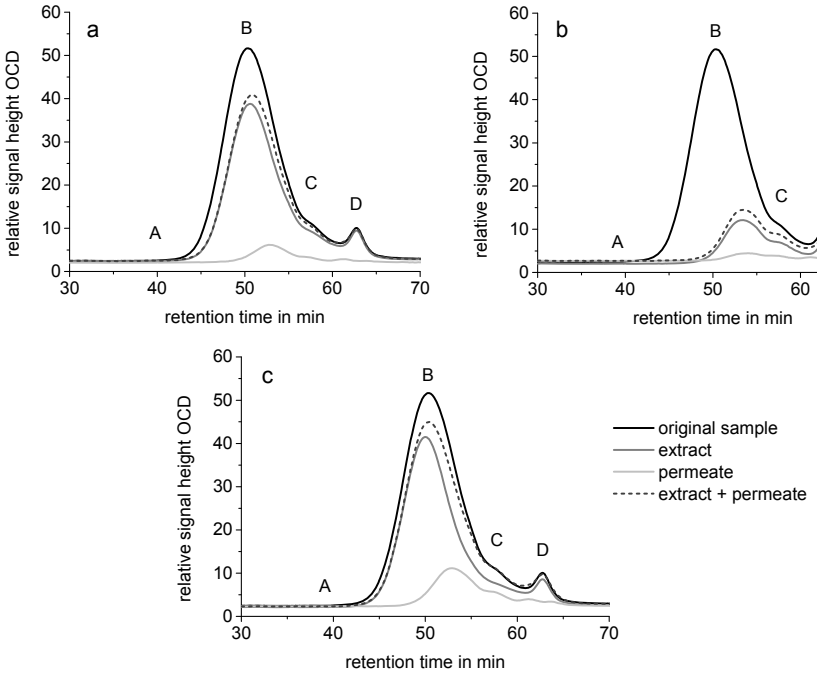

Fig. S1. SEC-OCD chromatograms of the original freshwater sample I and the extracts and permeates of a: PPL, b: HLB and c: C18, A: biopolymers, B: humic substances, C: building blocks, D: low molecular weight acids.

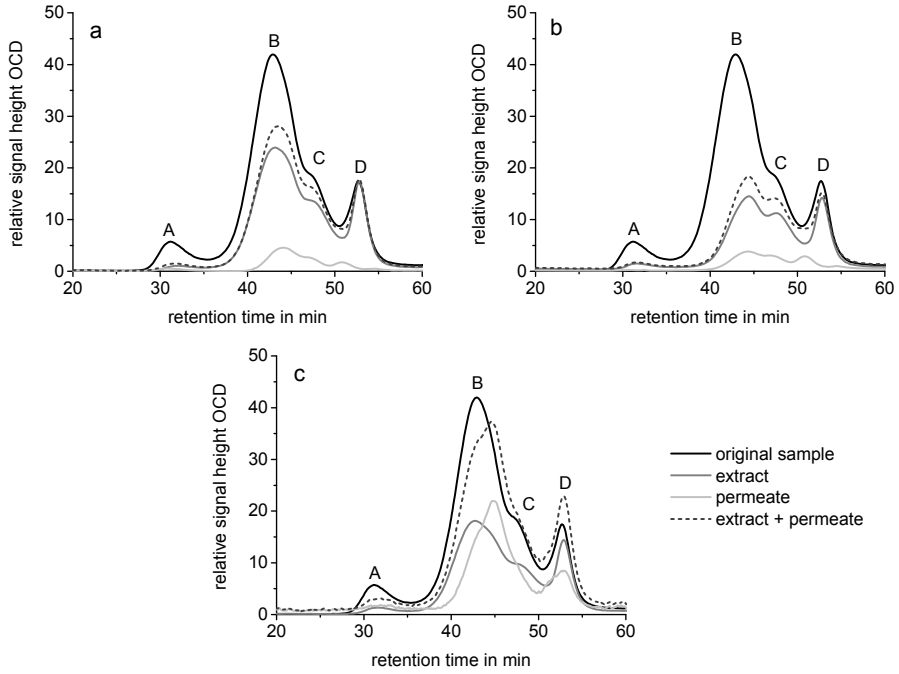

Fig. S2. SEC-OCD chromatograms of the original freshwater sample II and the extracts and permeates of a: PPL, b: HLB and c: C18, A: biopolymers, B: humic substances, C: building blocks, D: low molecular weight acids.

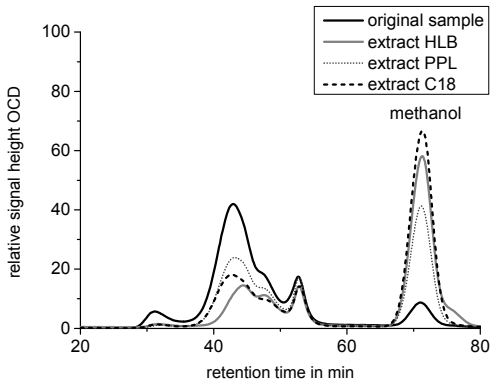

Fig. S3. SEC-OCD chromatogram of the original sample II and of the extracts of the HLB, the PPL and the C18 cartridge containing the methanol peak ($t_R \approx 70$ min).

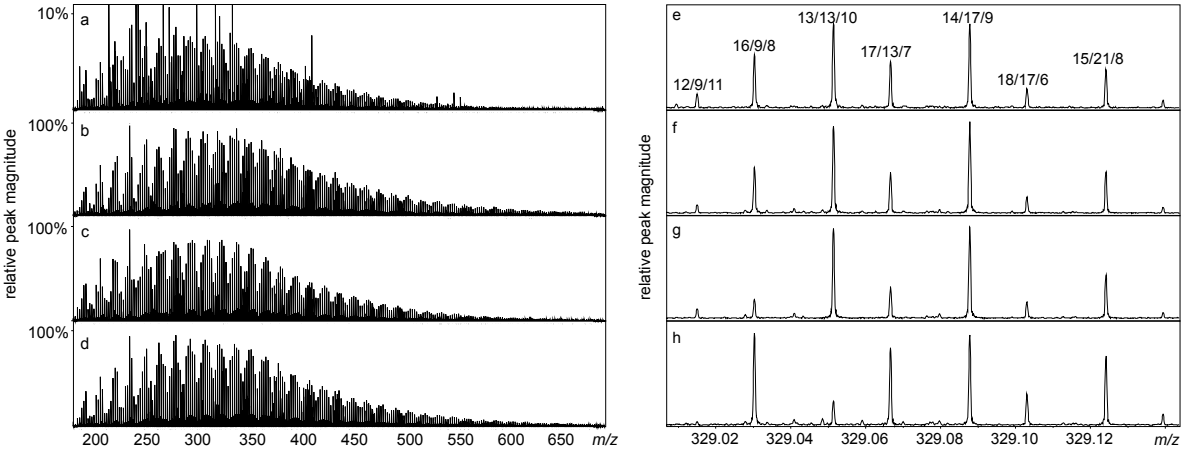

Fig. S4. FT-ICR mass spectra of the original sample III and the extracts of the different SPE cartridges, full spectrum (left) and nominal mass 329 (right), a, e: original sample, b, f: PPL extract, c, g: HLB extract, d, h: C18 extract. Numbers at peak tops denote the numbers of C/H/O in the respective molecular anions.

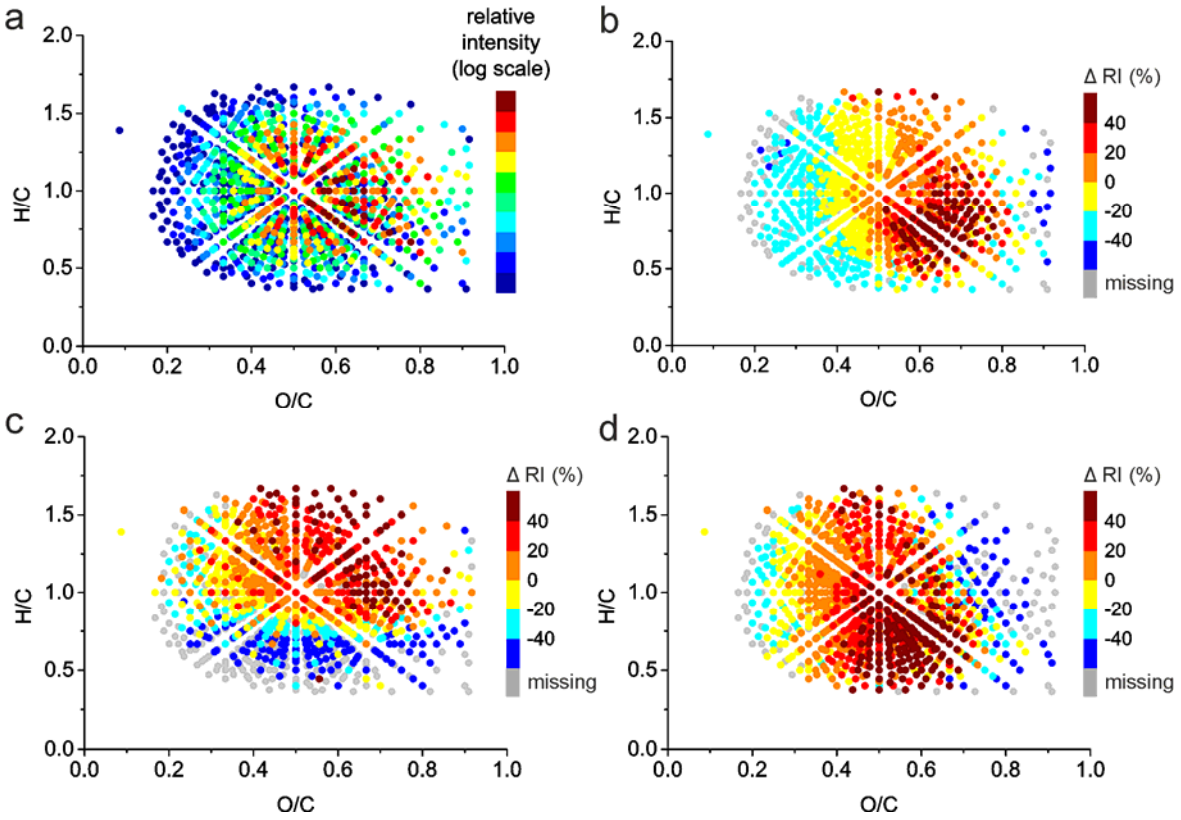

Fig. S5. Van Krevelen diagrams of molecular formulae (only $C_xH_yO_z$) in a: the original sample III, b: the PPL extract, c: the HLB extract, d: the C18 extract. The Δ RI was calculated relative to the original sample III (see eqn (2) in the main text).

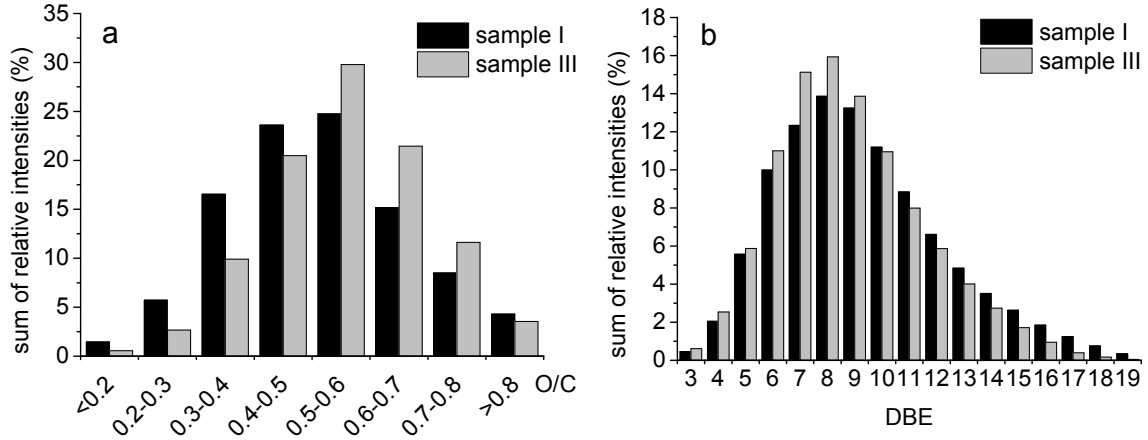

Fig. S6. Frequency distribution of the O/C ratios (a) and the DBE values (b) of sample I and III, based on the original samples.