Optimization of a Simple, Effective, and Greener methodology for Polycyclic Aromatic Hydrocarbons extraction from Human Adipose Tissue - Supplementary Material

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Table SM1 – PAHs content of each standard solution (S) prepared (µg/L).

PAHs	Concentration (µg/L)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
B[a]P (reference)	0.13	0.25	0.50	1.25	2.51	5.01	7.52	10.03	15.04	20.06
Naph	1.30	2.52	5.01	12.55	25.07	50.15	75.22	100.30	150.45	200.60
Acy	73.88	100.07	148.63	171.61	196.67	298.30	399.93	497.60	597.90	700.30
Ace	1.30	2.52	5.01	12.55	25.07	50.15	75.22	100.30	150.45	200.60
Flu	0.26	0.50	0.99	2.48	4.95	9.89	14.84	19.79	29.68	39.58
Phe	0.13	0.25	0.50	1.25	2.50	5.00	7.51	10.01	15.01	20.01
Ant	0.13	0.25	0.50	1.25	2.50	5.01	7.51	10.02	15.03	20.04
Fln	0.26	0.50	0.99	2.47	4.93	9.86	14.80	19.73	29.59	39.46
Pyr	0.13	0.24	0.48	1.21	2.42	4.84	7.27	9.69	14.53	19.38
B[a]A	0.13	0.25	0.49	1.23	2.46	4.92	7.38	9.84	14.76	19.68
Chry	0.13	0.25	0.50	1.24	2.48	4.95	7.43	9.91	14.86	19.82
B[b]Ft + B[j]Ft	0.52	1.00	2.00	5.01	10.02	20.04	30.06	40.08	60.12	80.16
B[k]Ft	0.13	0.25	0.50	1.25	2.49	4.99	7.48	9.98	14.97	19.96
DB[a,l]P	0.26	0.50	1.00	2.50	5.00	10.00	15.00	20.00	30.00	40.00
DB[a,h]A	0.26	0.50	1.00	2.50	5.00	10.00	15.00	20.00	30.00	40.00
B[g,h,i]P	0.26	0.50	0.99	2.49	4.97	9.95	14.92	19.90	29.85	39.80
InP	0.13	0.25	0.50	1.26	2.51	5.02	7.53	10.04	15.06	20.08

acenaphthene (Ace); acenaphthylene (Acy); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[k]Ft); chrysene (Chry); dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH); pyrene (Pyr); S – standard
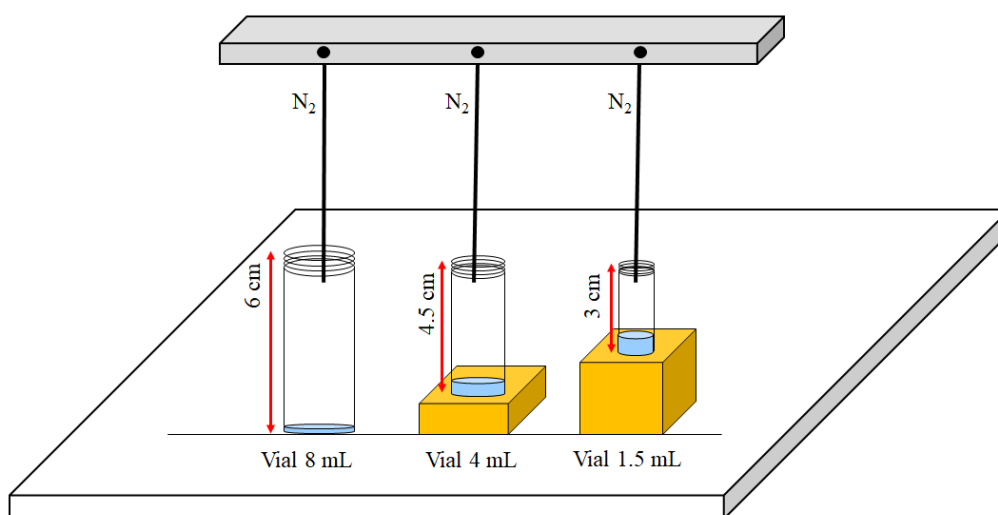

Figure SM1 – Diagram of nitrogen (N_2) evaporation step of PAHs standard mixture S8 in different capacity glass vials (section 2.3.3).
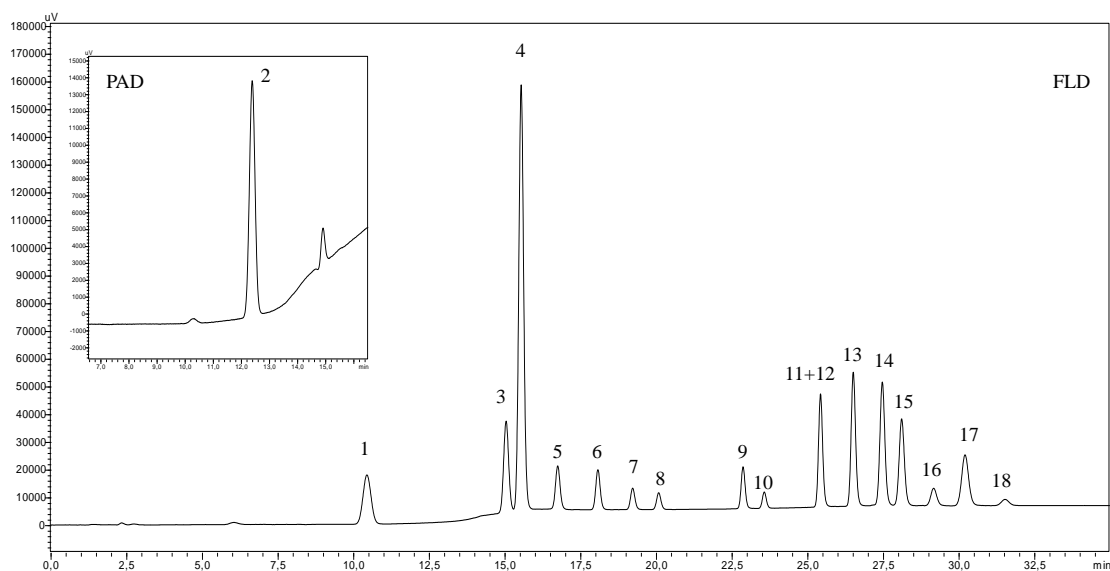
Figure SM2 – HPLC chromatogram for PAHs standard mixture S8 [1-naphthalene (Naph); 2-acenaphthylene (Acy); 3-acenaphthene (Ace); 4-fluorene (Flu); 5-phenanthrene (Phe); 6-anthracene (Ant); 7-fluoranthene (Fln); 8-pyrene (Pyr); 9-benz[a]anthracene (B[a]A); 10-chrysene (Chry); 11+12-benzo[b]fluoranthene (B[b]Ft + benzo[j]fluoranthene (B[j]Ft); 13-benzo[k]fluoranthene (B[k]Ft); 14-benzo[a]pyrene (B[a]P); 15-dibenzo[a,l]pyrene (DB[a,l]P); 16-dibenz[a,h]anthracene (DB[a,h]A); 17-benzo[g,h,i]perylene (B[g,h,i]P); and 18-indeno[1,2,3-cd]pyrene (InP)]; FLD – fluorescence detector; PDA- photodiode array detector.
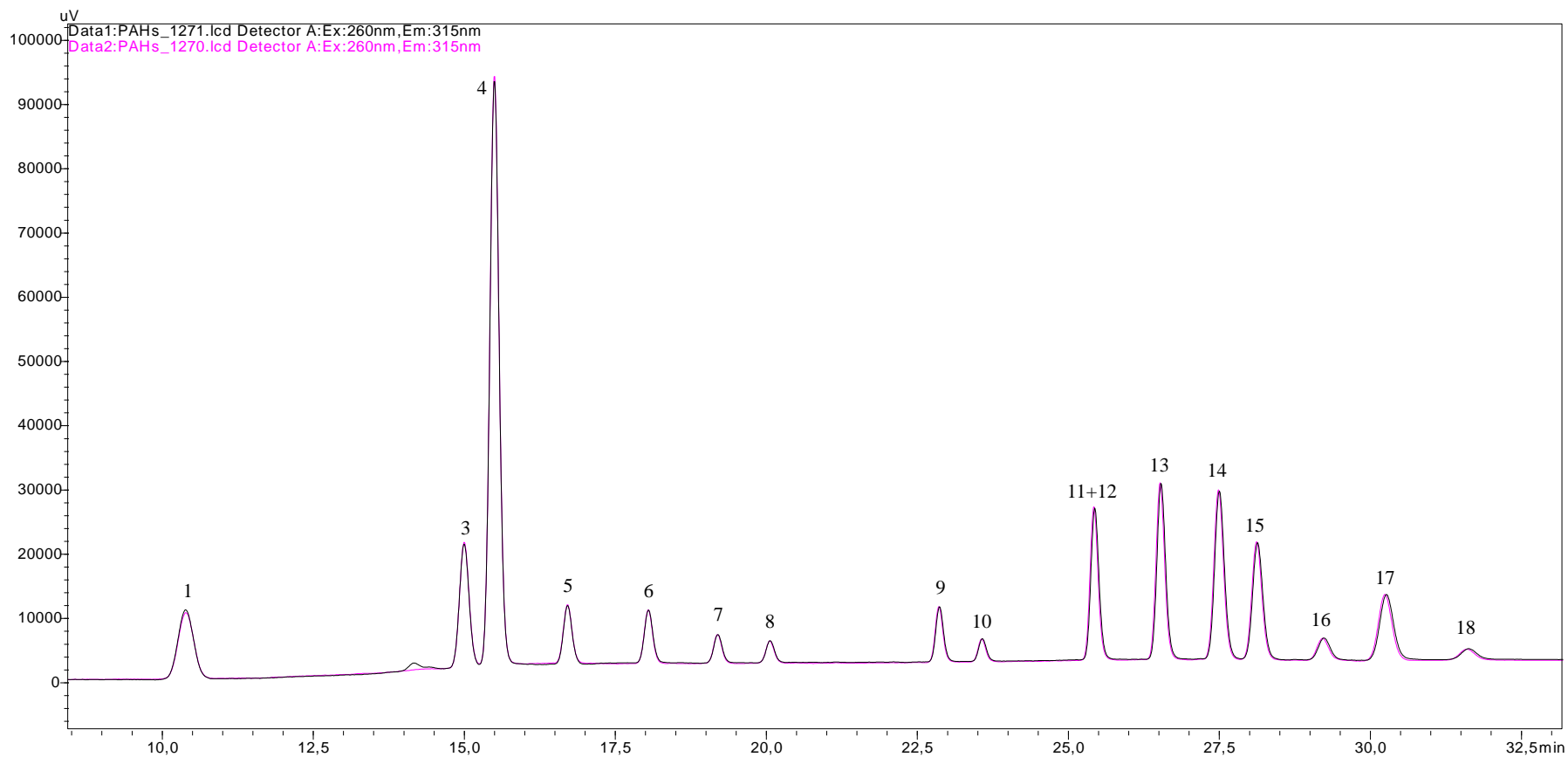
Figure SM3– Overlay of HPLC-FLD chromatograms of PAHs standard mixture S8 in two different conditions: S8 without being filtered (black) and S8 filtered (pink). Legend: 1-naphthalene (Naph); 3-acenaphthene (Ace); 4-fluorene (Flu); 5-phenanthrene (Phe); 6-anthracene (Ant); 7-fluoranthene (Fln); 8-pyrene (Pyr); 9-benz[a]anthracene (B[a]A); 10-chrysene (Chry); 11+12-benzo[b]fluoranthene (B[b]Ft) + benzo[j]fluoranthene (B[j]Ft); 13-benzo[k]fluoranthene (B[k]Ft); 14-benzo[a]pyrene (B[a]P); 15-dibenzo[a,l]pyrene (DB[a,l]P); 16-dibenz[a,h]anthracene (DB[a,h]A); 17-benzo[g,h,i]perylene (B[g,h,i]P); and 18-indeno[1,2,3-cd]pyrene (InP).

Table SM2– Redissolution of PAHs after evaporation in different solvents, expressed as loss of PAHs (%).

PAHs	PAHs loss (%)					
	Solvent					
	100% Ultra-pure water	75:25% Ultra-pure water-ACN	50:50% Ultra-pure water-ACN	25:75% Ultra-pure water-ACN	100% ACN	100% ACN with 0.1% formic acid
Naph	100	100	100	100	100	100
Acy	100	100	100	100	97.6	100*
Ace	100	100	100	100	97.1	97.7
Flu	99.8	99.2	99.2	98.4	92.1	98.7**
Phe	98.5	92.5	92.7	75.2	55.2	92.4****
Ant	98.7	92.6	92.1	75.4	54.5	92.0****
Fln	90.5	40.7	33.5	22.0	9.1	29.3***
Pyr	89.7	33.6	23.7	14.6	6.5	22.6***
B[a]A	97.3	15.5	3.5	3.0	3.4	2.9
Chry	95.7	16.0	3.0	2.0	-1.3	4.9**
B[b]Ft + B[j]Ft	99.4	15.2	1.0	2.0	0.2	0.4
B[k]Ft	99.2	16.5	1.6	2.2	0.9	0.3
B[a]P	100	15.7	1.5	1.4	0.0	0.7
DB[a,l]P	100	26.3	2.3	1.7	-0.7	0.7
DB[a,h]A	100	19.4	1.2	0.6	-4.1	-2.5*
B[g,h,i]P	100	18.7	1.0	1.0	-1.4	2.2**
InP	100	21.3	2.5	1.0	2.0	-3.9**

Statistical significance was evaluated by Students t-test. Significantly difference between 100% ACN with and without formic acid, $*p < 0.05$, $** p < 0.01$, $*** p < 0.001$, $**** p < 0.0001$. Acenaphthene (Ace); acenaphthylene (Acy); acetonitrile (ACN); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[k]Ft); chrysene (Chry); dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH) and pyrene (Pyr).
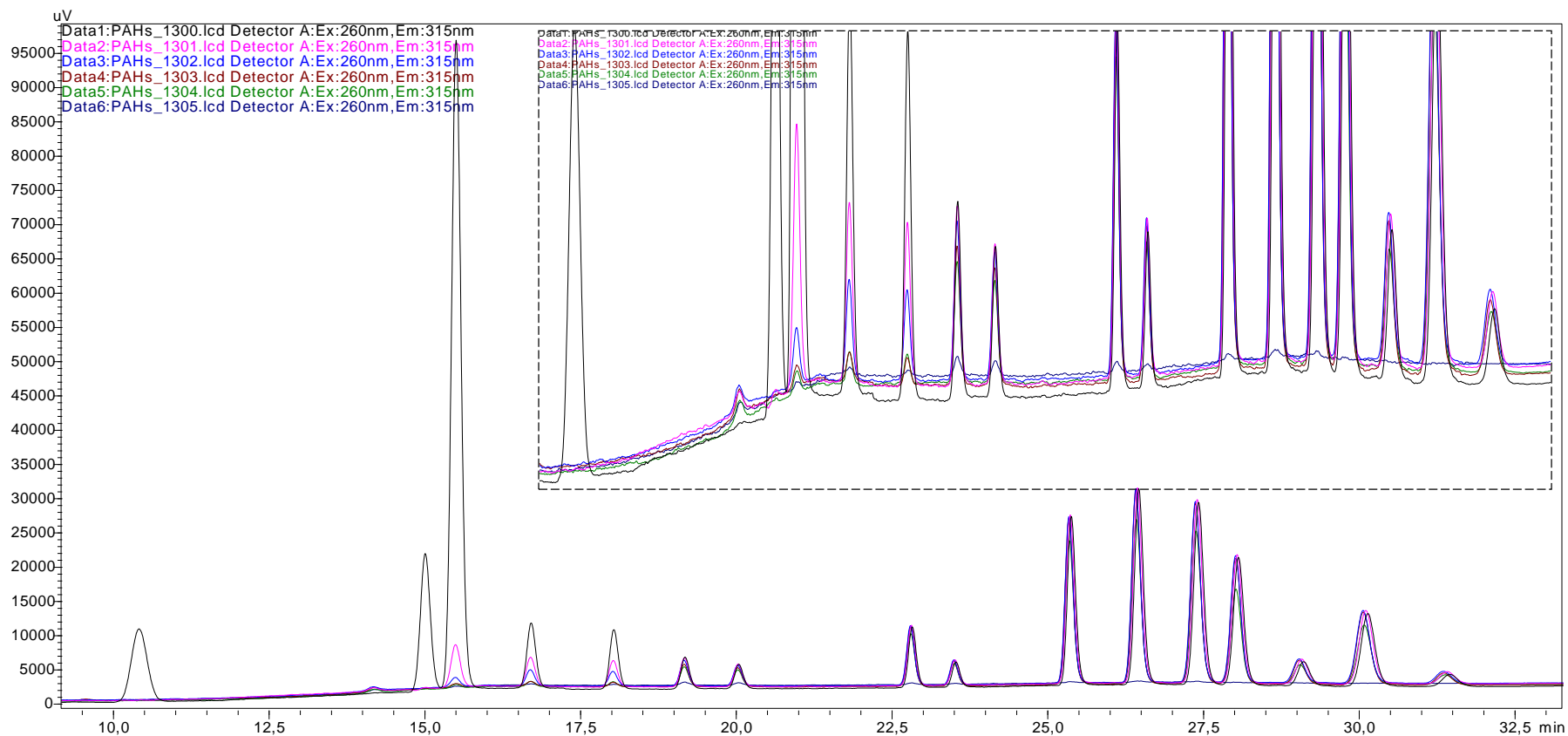
Figure SM4– Overlay of HPLC-FLD chromatograms of PAHs standard mixture after evaporation and redissolution in different solvents: S8 without being evaporated (black), S8 evaporated and redissolved in 100% ACN (pink), S8 evaporated and redissolved in 75% ACN/25% ultra-pure water (blue), S8 evaporated and redissolved in 50% ACN/50% ultra-pure water (brown), S8 evaporated and redissolved in 25% ACN/75% ultra-pure water (green) and S8 evaporated and redissolved in 100% ultra-pure water (dark blue). Note: Enlarged chromatogram area in dashed squares.
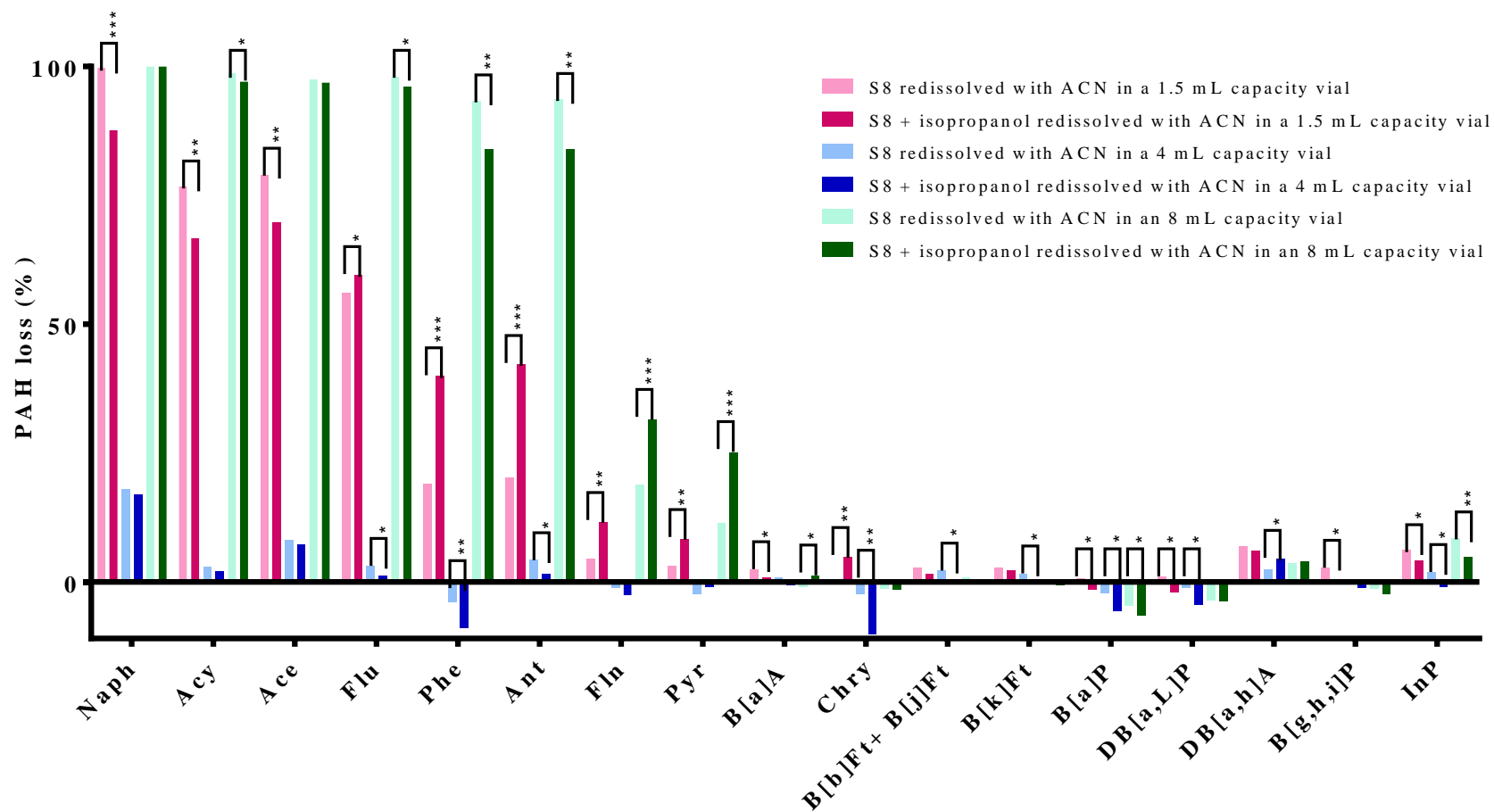
Figure SM5– Loss of PAHs (%) during evaporation in different capacity vials with and without the use of a keeper. Standard error of mean (SEM). Statistical significance was evaluated by Students t-test. Significantly difference between vials with and without the use of a keeper, *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Legend: Acenaphthene (Ace); acenaphthylene (Acy); acetonitrile (ACN); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[k]Ft); chrysene (Chry); dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH) and pyrene (Pyr).
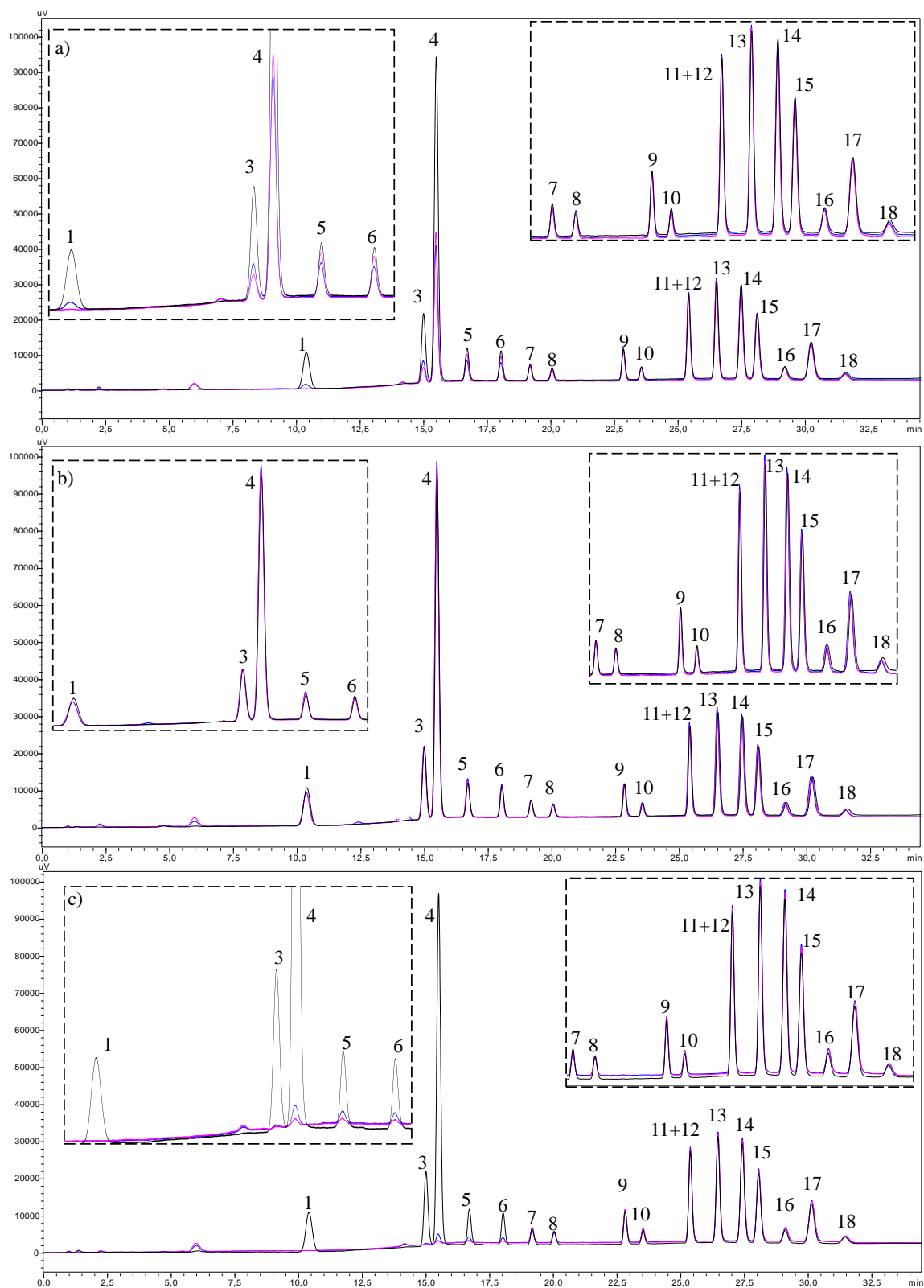
Figure SM6 – Overlay of HPLC-FLD chromatograms of PAHs standard mixture S8 in three different conditions: S8 without being evaporated (black), S8 evaporated (pink), or isopropanol and S8 evaporated (blue) in a) 1.5 mL capacity vial, b) 4 mL capacity vial, or c) 8 mL capacity vial.

Note: Enlarged chromatogram area in dashed squares. Legend: 1-naphthalene (Naph); 3-acenaphthene (Ace); 4-fluorene (Flu); 5-phenanthrene (Phe); 6-anthracene (Ant); 7-fluoranthene (Fln); 8-pyrene (Pyr); 9-benz[a]anthracene (B[a]A); 10-chrysene (Chry); 11+12-benzo[b]fluoranthene (B[b]Ft) + benzo[j]fluoranthene (B[j]Ft); 13-benzo[k]fluoranthene (B[k]Ft); 14-benzo[a]pyrene (B[a]P); 15-dibenzo[a,l]pyrene (DB[a,l]P); 16-dibenz[a,h]anthracene (DB[a,h]A); 17-benzo[g,h,i]perylene (B[g,h,i]P); and 18-indeno[1,2,3-cd]pyrene (InP).

Figure SM7 –Overlay of HPLC-PDA chromatograms of acenaphthylene (Acy) standard S8 in three different conditions: S8 without being evaporated (black), S8 evaporated (pink), or isopropanol and S8 evaporated (blue) in a) 1.5 mL capacity vial, b) 4 mL capacity vial, and c) 8 mL capacity vial.

Figure SM8 – Effect on PAHs recoveries (%±SEM) of sample preparation and heat in UAE. Standard error of mean (SEM). Statistical significance was evaluated by Students t-test. Significantly difference between UAE with adipose tissue, *$p < 0.05$.

Table SM3- PAHs (%) recoveries after fatty sample clean-up with additionally C18 and Z-Sep.

PAHs	Recovery (%)
Naph	16.3
Acy	0.0
Ace	66.3
Flu	84.9
Phe	53.1
Ant	81.8
Fln	14.1
Pyr	2.9
B[a]A	103.4
Chry	68.9
B[b]Ft + B[j]Ft	73.8
B[k]Ft	81.3
B[a]P	70.8
DB[a,l]P	78.5
DB[a,h]A	57.0
B[g,h,i]P	63.6
InP	59.5

acenaphthene (Ace); acenaphthylene (Acy); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[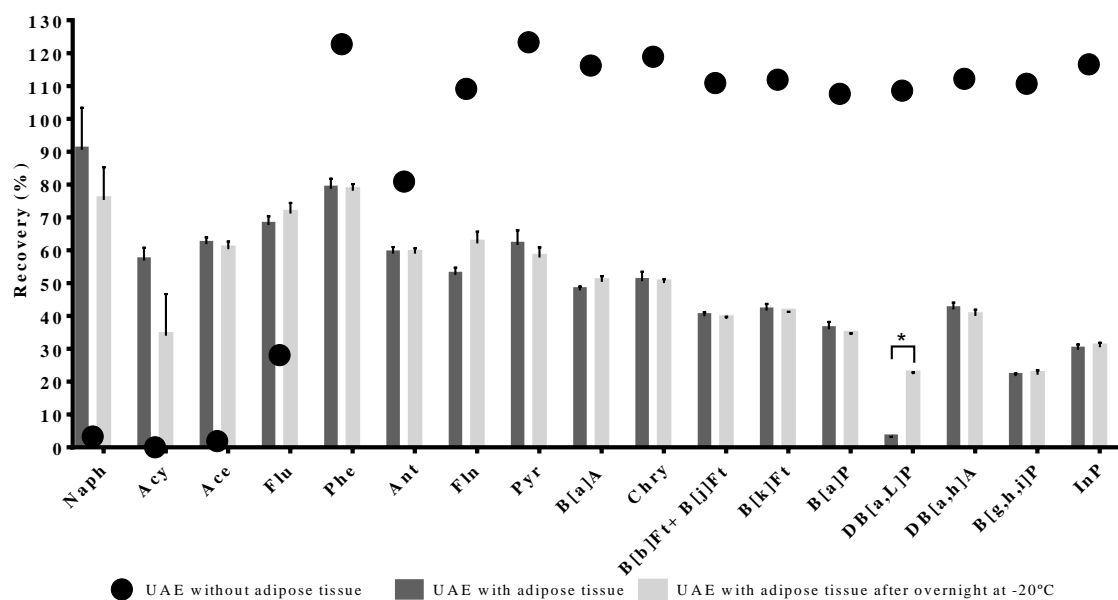k]Ft); chrysene (Chry); dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH) and pyrene (Pyr).

Table SM4 – PAHs (%) recoveries with different amounts of adipose tissue extract.

PAHs	Amount of adipose tissue extract (mL)		
	3.5	2.0	0.2
	Amount of fat in adipose tissue extract (g)		
	0.006	0.004	0.0005
	Recovery (%)		
Naph	110.7	80.1	52.0
Acy	46.4	60.0	72.2
Ace	58.8	64.7	74.3
Flu	69.6	73.2	79.6
Phe	80.0	73.4	81.4
Ant	62.6	67.0	77.8
Fln	56.2	64.6	74.6
Pyr	62.9	65.5	70.7
B[a]A	39.9	60.4	72.0
Chry	44.1	63.6	71.5
B[b]Ft + B[j]Ft	46.7	52.7	63.4
B[k]Ft	46.6	52.6	63.4
B[a]P	34.4	46.3	57.4
DB[a,l]P	25.9	36.8	44.5
DB[a,h]A	37.3	60.4	61.4
B[g,h,i]P	26.1	37.1	47.6
InP	33.6	39.8	52.1
Average	51.9	58.7	65.6

acenaphthene (Ace); acenaphthylene (Acy); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[k]Ft); chrysene (Chry); dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH) and pyrene (Pyr).

Figure SM9- Overlay of HPLC-FLD chromatograms of PAHs standard mixture S8 in three different conditions and adipose tissue extracts with different concentration factors (CF): S8 standard (black), CF=1 (pink), CF = 5 (blue), CF = 10 (brown), CF =15 (green), CF= 20 (dark blue) and CF = 25 (light green). Note: Enlarged chromatogram area in dashed squares.

Figure SM10 – Overlay HPLC-FLD and HPLC-PDA chromatograms of PAHs standard mixture S8 (black) and adipose tissue extracts (pink) in different exchange solvents: a) ACN, b) ethyl acetate, c) acetone, d) toluene, and e) DCM.

Table SM5 – Signal suppression or enhancement (%) for PAHs with different exchange solvents.

| | Signal suppression/ enhancement (%) | | | | | | | | | |
| | Solvent exchange | | | | | | | | | |
PAHs	ACN	Ethyl acetate	Acetone	Toluene	DCM	ACN/Ethyl Acetate (50:50%, v/v)	ACN/Acetone (50:50%, v/v)	ACN/Toluene (50:50%, v/v)	ACN/DCM (50:50%, v/v)	ACN/Ethyl Acetate (25:75%, v/v)
Naph	2.2	-36.9	-34.6	-35.7	-34.6	-22.6	-7.8	-37.8	-36.0	-35.6
Acy	-0.1	-23.3	-17.7	-16.7	-13.4	-13.8	-12.3	-30.8	-18.9	-12.9
Ace	111.5	56.2	62.5	50.9	71.4	80.2	102.4	54.5	62.7	-27.2
Flu	-58.6	-67.6	-66.9	-71.2	-70.1	-62.9	-60.5	-70.9	-64.5	-27.0
Phe	-4.6	-25.4	-26.0	-29.3	-24.2	-15.2	-10.9	-26.4	-23.8	-20.6
Ant	-6.3	-32.3	-31.9	-32.0	-29.1	-18.8	-11.6	-31.1	-31.5	-23.9
Fln	-0.7	-27.0	-23.9	-26.6	-24.8	-14.5	-6.0	-27.1	-26.0	-22.9
Pyr	-0.3	-30.5	-28.5	-30.1	-30.5	-18.3	-6.8	-31.1	-29.3	-26.3
B[a]A	1.3	-33.4	-28.2	-33.6	-33.3	-20.4	-4.9	-35.1	-34.7	-30.3
Chry	-2.3	-36.8	-34.5	-35.4	-36.1	-23.0	-9.3	-37.1	-36.9	-33.3
B[b]Ft + B[j]Ft	-0.2	-36.6	-35.6	-35.1	-36.1	-21.0	-7.9	-36.0	-36.5	-33.3
B[k]Ft	-0.1	-45.0	-42.4	-42.1	-43.2	-28.6	-10.6	-44.2	-44.2	-41.5
B[a]P	0.0	-45.0	-42.3	-41.9	-43.4	-28.3	-10.2	-44.0	-44.4	-41.2
DB[a,l]P	0.7	-50.7	-48.5	-47.3	-49.0	-33.1	-12.2	-49.8	-49.9	-46.3
DB[a,h]A	1.9	-62.6	-62.1	-58.3	-62.6	-47.3	-19.5	-63.1	-63.4	-62.3
B[g,h,i]P	0.8	-47.7	-46.0	-43.0	-45.4	-29.9	-9.8	-47.0	-47.2	-44.4
InP	-0.2	-62.2	-59.7	-57.4	-61.1	-44.3	-18.3	-60.8	-62.0	-58.2

acenaphthene (Ace); acenaphthylene (Acy); acetonitrile (ACN); anthracene (Ant); benz[a]anthracene (B[a]A); benzo[a]pyrene (B[a]P); benzo[b]fluoranthene (B[b]Ft); benzo[g,h,i]perylene (B[g,h,i]P); benzo[j]fluoranthene (B[j]Ft); benzo[k]fluoranthene (B[k]Ft); chrysene (Chry); DCM – dichloromethane; dibenz[a,h]anthracene (DB[a,h]A); dibenzo[a,l]pyrene (DB[a,l]P); fluoranthene (Fln); fluorene (Flu); indeno[1,2,3-cd]pyrene (InP); naphthalene (Naph); phenanthrene (Phe); polycyclic aromatic hydrocarbon (PAH) and pyrene (Pyr).
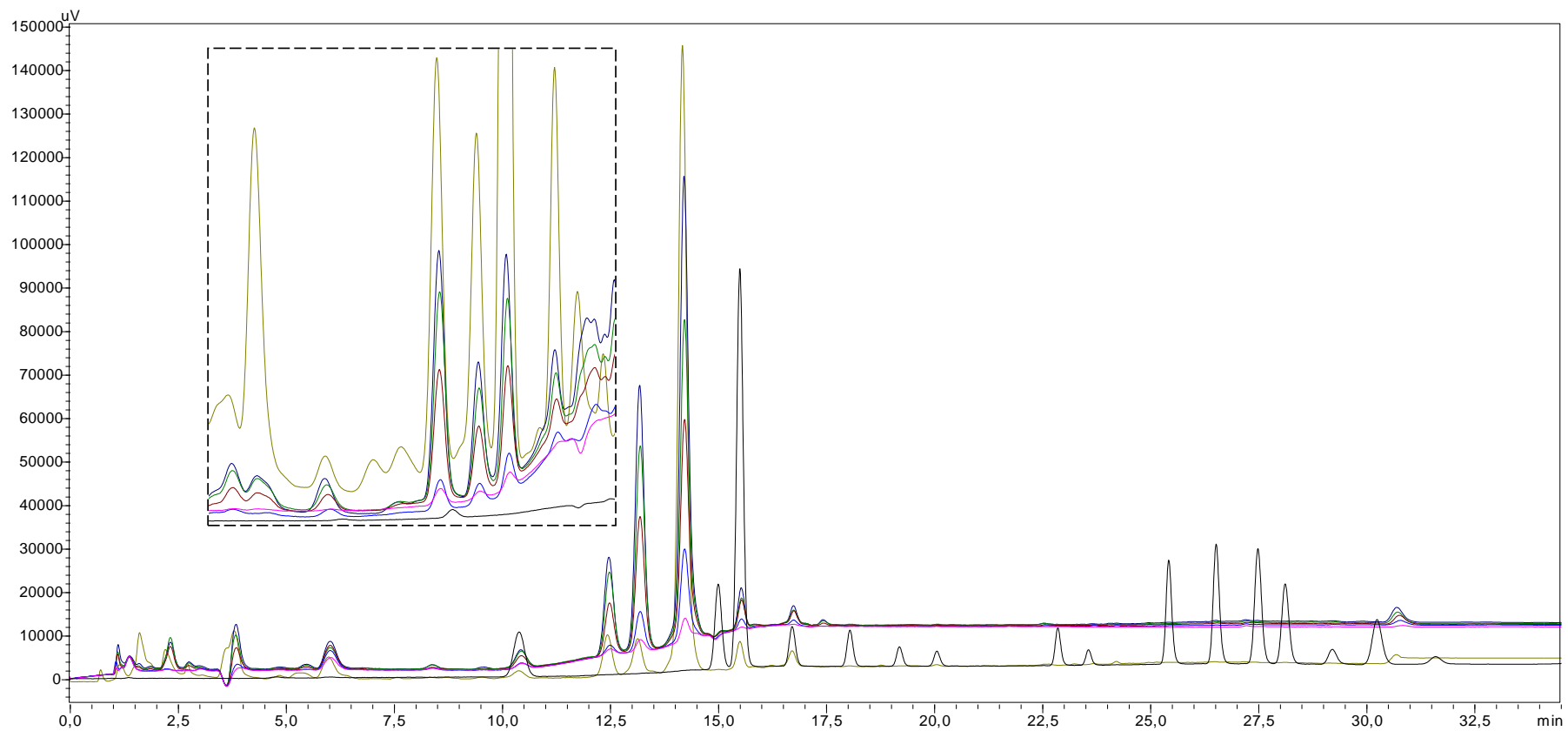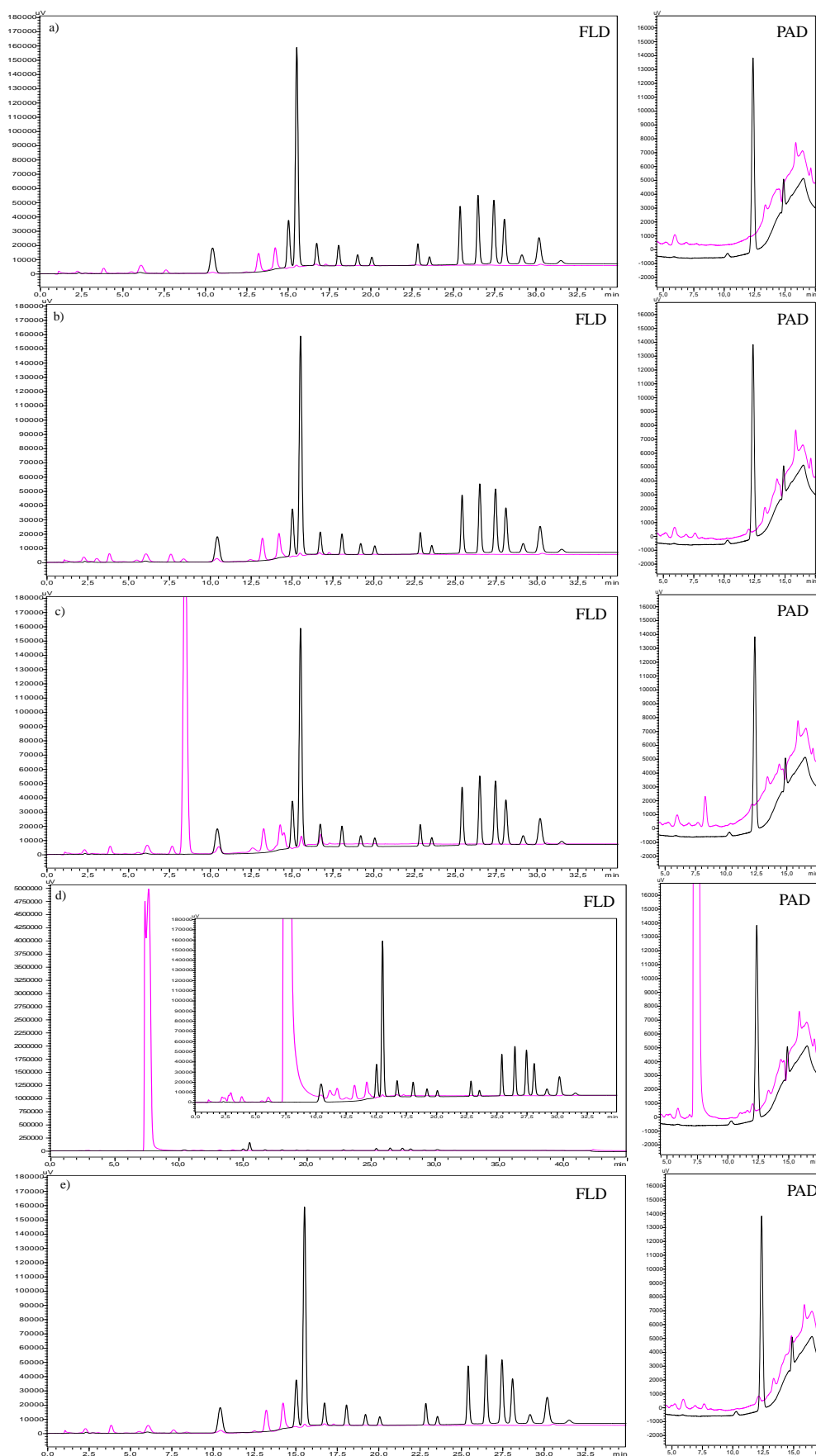
Table SM6. Methods eco-scale score for the presented study and other existent studies for PAHs determination in adipose tissue.

Description/Reference	Penalty Points								
	Present study (Method 1)[1]	Present study (Method 2)[2]	[21]	[22,23]	[24]	[25,26]	[27]	[28,29]	[30]
ACN	2 (1.14 mL)	2 (0.14 mL)	2 (3.5 mL)	-	4 (70 mL)	-	-	-	-
Alumina	-		-	-	-	-	-	-	0 (6g)
Analytes standard solution	4		4	4	4	4	4	4	4
Bio-Beads S–X3 (styrene divinylbenzene)	-		-	0	0	-	-	0 (60g)	-
CCl_4	-		8 (50 µL)	-	-	-	-	-	-
DCM	-		-	3 (425 mL)	-	-	-	-	-
Diethyl ether	-		-	-	-	-	-	12 (162 mL)	8
Dimethylsulfoxide	-		-	-	-	-	-	-	8
Ethanol	-		-	-	-	-	-	-	4
Ethyl Acetate	-	4 (1 mL)	-	-	8 (12 mL)	-	-	-	-
Florisil	-		-	-	-	-	-	0 (12.5 g)	-
n-Hexane	8 (6 mL)		-	24 (225 mL)	16 (12 mL)	16 (52 mL)	16 (24.5 mL)	32 (1593 mL)	32 (900 mL)
KOH	-		-	-	-	-	-	-	4
Methylene Chloride	-		-	-	-	24 (425 mL)	-	32 (1411 mL)	-
Na_2SO_4	-		-	0	0 (15 g)	0 (10 g)	0 (5-10 g)	0 (5-10 g)	0
NaCl	-		0 (9 mL 5%)	-	-	-	-	-	0 (100 mL 15%)
n-nonane	-		-	6 (1 mL)	-	-	-	-	-
Silica gel	-		-	0 (0.5 g)	-	-	-	-	0 (5g)
Water	-		0 (3 mL)	-	-	-	-	-	0 (300 mL)

(Row group label, vertical: Chemicals)

[1] Use of ACN as solvent exchange; [2] Method 2- Use of ACN/ethyl acetate (25:75%, v/v) as solvent exchange. Acetonitrile (ACN); dichloromethane (DCM) and polycyclic aromatic hydrocarbon (PAH).

Table SM6. Methods eco-scale score for the presented study and other existent studies for PAHs determination in adipose tissue (continuation).

Description/Reference		Present study (Method 1)[1]	Present study (Method 2)[2]	Penalty Points 21	22,23	24	25,26	27	28,29	30
Instruments	Centrifuge	0	0	-	0	-	-	-	-
	GC-MS	-	2	2	2	2	-	2	-
	GC-TOF MS	-	-	-	-	-	2	-	-
	Heater	-	-	-	-	-	2	-	-
	Homogeniser	-	-	-	0	-	-	0	-
	Kuderna-Danish evaporator	-	-	-	-	1	-	1	-
	LC-FLD	-	-	-	-	-	-	-	1
	LC-FLD-PDA	1	-	-	-	-	-	-	-
	Needle evaporator	0	-	-	-	-	0	0	-
	Rotary evaporator	-	-	-	2	-	-	-	-
	Soxhlet	-	-	2	-	2	-	-	-
	Ultrasonic processor	0	-	-	-	-	-	-	-
	Vortex	0	-	-	-	-	0	-	-
Others	Occupational hazard	3	3	3	3	0	6	3	3
	Waste	8	8	5	5	8	3	8	8
	Total penalty points	26	30	27	49	44	57	33	94	72
	Analytical Eco-Scale total score	**74**	**70**	**73**	**51**	**56**	**43**	**67**	**6**	**28**

[1] Use of ACN as solvent exchange; [2] Method 2- Use of ACN/ethyl acetate (25:75%, v/v) as solvent exchange. Gas chromatography (GC); fluorescence detector (FLD); liquid chromatography (LC); mass spectrometry (MS); photodiode array detector (PDA); polycyclic aromatic hydrocarbon (PAH); time – of- flight (TOF).