Supporting Information

A new generation of Solid Phase Microextraction coatings for complementary separation approaches: a step toward comprehensive metabolomics and multiresidue analyses

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Section 1 – LC-MS analysis

Chromatographic separation of the analytes under study was achieved by a Discovery HS F5 column (100 mm × 2.1 mm i.d., 3 µm; Supelco, Bellefonte, PA, USA) using a 0.3 mL min^{-1} flow rate in a ternary gradient elution with water (solvent A) and acetonitrile (solvent B) and methanol (solvent C), acidified with FA (0.1% *v/v*). The gradient employed was as follows: the initial composition of solvents was 5% B and 5% C; this composition was held for the first 0.5 min, then raised to 50% B and 50% C. After 6.5 min, the composition of B was gradually decreased to 25% and the composition of C increased to 75% within a 5 min period. This composition was kept for 3.5 min, then restored to initial conditions within 0.2 min, and finally held for an additional 2 min to ensure re-equilibration of the column. The injection volume for all standards and samples was 10 µL in full loop mode. The tray temperature of the autosampler was maintained at 5 ºC while the column oven compartment temperature was set at 35 ºC. Instrumental performance throughout analysis was monitored using quality control samples (QC) consisted of 50 ng mL^{-1} of compounds under study. QC samples were injected periodically throughout the sequences, including at the beginning and end of each sequence. Eluted analytes from the column were introduced to mass spectrometry after their ionization using an electrospray ionization probe (ESI) operated in positive mode. Parameters used for ionization of analytes in ESI are reported in Table S2 (Supporting Information). The mass resolution and automatic gain control target were set at 100,000 FWHM for *m/z* 200 and at a balanced range (1 x 10^6 ions), respectively. Instrument calibration was performed daily using the positive calibration solution MSCAL5, consisting of caffeine, tetrapeptide "Met-Arg-Phe-Ala", and ultramark 1621. Instrument settings, data acquisition, and processing were carried out by Xcalibur software (version 2.2) provided by Thermo Scientific.

Section 2 – GC-MS analysis

The capillary column used for chromatographic separation was an Agilent J&W HP-5 (30 m, 0.25 mm i.d., 0.25 µm film thickness). The column temperature program was initially set at 35 °C for 6 min, ramped at a rate of 10 °C/min to 140 °C, then ramped at 20 ºC/min to 270 ºC, where it was held for 2 min, giving a total run time of 24 min. Helium (purity level 99.999%)

was used as carrier gas and its flow set at 1.2 mL/min. The mass spectrometer working conditions were: electron ionization (EI) 70eV; mass range 50-350 m/z; ion source temperature: 230 °C; quadrupole temperature: 150 °C; transfer line temperature: 280°C. QC analyses to test instrumental response were run daily.

Section 3- Desorption solvent selection

The optimal desorption solvent should quantitatively desorb all of the extracted analytes in a reasonable time with minimum to no carry-over. For this purpose, four different solvents were prepared and tested, namely Solvent A: ACN/MeOH (80/20, v/v) with 0.1% (v/v) FA; Solvent B: ACN/H_2O(80/20, v/v) with 0.1% (v/v) FA; Solvent C: hexane; and Solvent D: ACN/MeOH/H_2O (40/40/20, v/v/v) with 0.1% (v/v) FA. This set of experiments was carried out using 2h as extraction and 2 h desorption time. The first desorption was followed by a second desorption at the same experimental conditions to evaluate whether carry-over occurred. Among the tested solvent mixtures, Solvent D was found to be the most suitable for the PTFE AF coatings, yielding the best desorption recoveries and lowest carry-over % (results not shown).

Section 4- Scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX) analysis

One of the most important parameters to consider when evaluating the biocompatibility of a SPME coating, is the occurrence of matrix components attachment on its surface after extraction. This factor indicates if the material inertness toward the matrix tested is sufficient to avoid bias during the extraction process. Among the various methods used to date for this kind of evaluation, SEM/EDX analysis represents a convenient tool to monitor changes in the chemical composition of the coating surface. Our evaluation consisted in performing SEM/EDX analysis on a new coating, never been in contact with whole blood, and a coating that was used 5 times for blood analysis. The abundance of elements that can indicate the accumulation of matrix constituents on the coating (such as proteins, red blood cells, phospholipids, etc..) as well as those characteristic of the coating composition, was monitored for both coatings. As shown in Figure S9, the abundance of all the elements monitored, namely carbon (C), nitrogen (N), oxygen (O), fluorine (F), phosphorus (P) and sulfur (S), did not show drastic variation for the

coatings monitored, indicating that the coating surface composition did not significantly change after multiple extractions in whole blood.

Section 5- Extraction coverage of PTFE AF/HLB fibers for LC and GC platforms

In order to show the applicability of the new PTFE AF/HLB coatings for integrated metabolomic studies, *in-fruit* extractions from apple were performed. For this purpose, extracts were analyzed with GC-ToF/MS and LC-HRMS instruments. GC and LC dedicated coatings were inserted in whole apple tissue and extraction took place for 60 min in static conditions. For extraction of LC-MS amenable compounds, fibers with coating dimensions of 7 mm (in length) and 45 µm (coating thickness) were used. The fibers were preconditioned in MeOH/H2O (50/50, v/v) for 30 min, then quickly rinsed with pure water. The rinsing step was followed by direct extraction from apple tissue for 60 min. Following the extraction, a quick rinsing step was performed using 300 µL of pure water, then extracted compounds were desorbed from the fiber in 100 µL of ACN/H_2O (50/50, *v/v*) using 1500 rpm agitation for 60 min. After desorption, extracts were analyzed by LC-HRMS in two separate runs, one with positive and the other with negative ionization modes using heated electrospray ionization (HESI). The data processing was performed using XCMS online. For extraction by GC-ToF/MS fibers with coating dimensions of 10 mm (in length) and 90 µm (coating thickness) were used. Prior extraction the coatings were conditioned at 250˚C for 30 minutes. After 60 min of extraction into the whole apple tissue the coatings were quickly rinsed in pure water for 10s and then desorbed at 250 ˚C for 15 minutes. Data processing was performed using ChromaTOF 1.4. The peak areas obtained for each feature of apple extracts were filtered in order to eliminate insignificant features. The filter parameters were as follows: any features present in fiber blanks was excluded. Results shown in Figure S11,

Figure S12 and Figure S13 reveal a good extraction coverage of the new coatings, over a wide range of molecular masses. For LC analysis 1267 features and 1829 features were obtained respectively by HESI(-)and HESI(+), in case of GC analysis 855 features were obtained by GC - MS employing Electron Impact Ionization (EI). The results presented show the potential of the new PTFE AF/HLB coatings for use in integrated metabolomics studies.

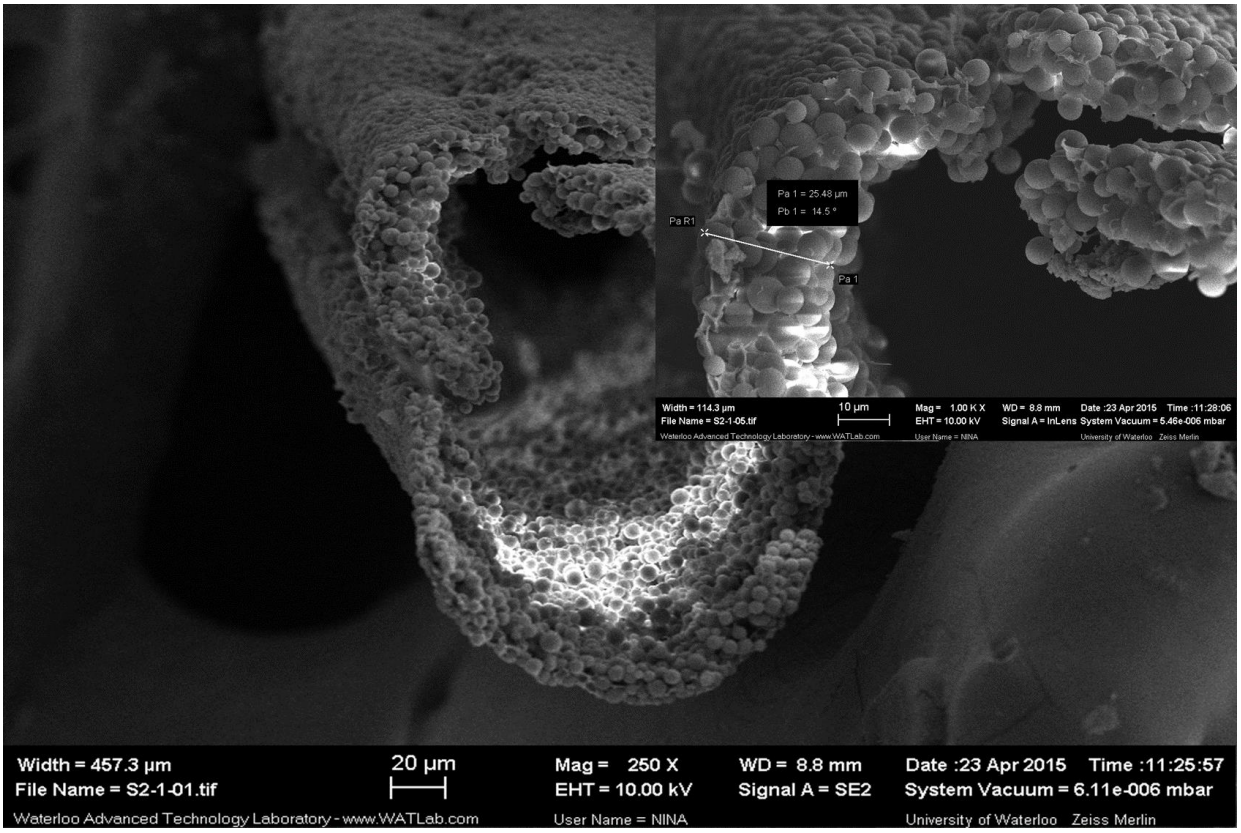

Figure S1: SEM image (x 250 magnification) of the PTFE AF/HLB coating obtained by dipping procedure. The calculated thickness was ~ 25 µm, obtained by 2 dipping cycles

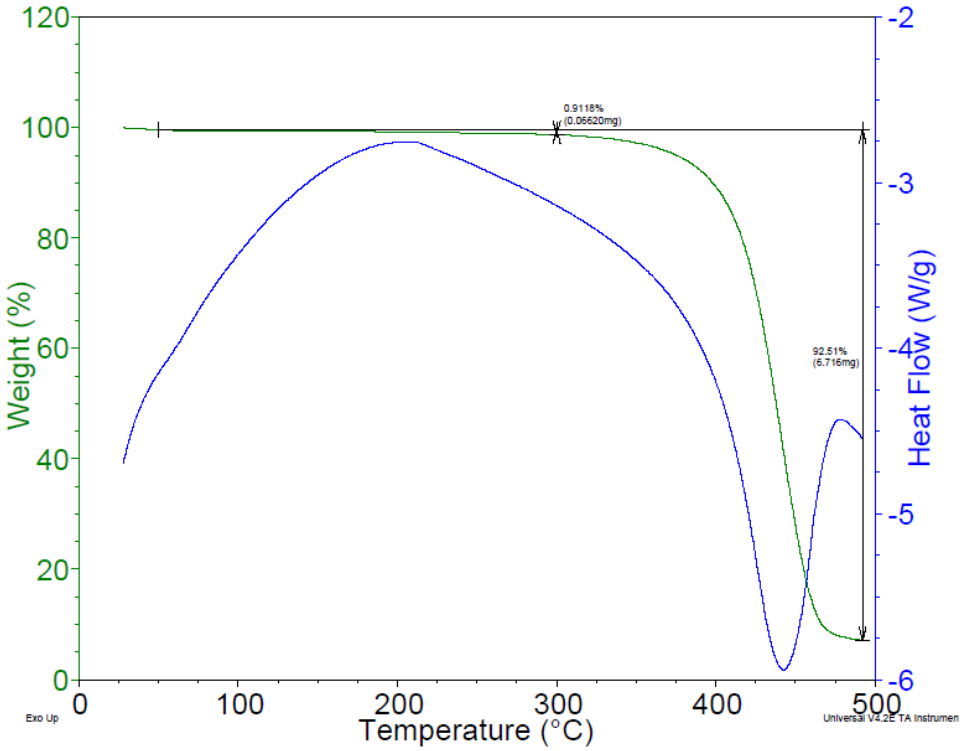

Figure S2: TGA thermograph of HLB particles in a temperature range from 25°C up to 500ºC. The left vertical axis refers to the total mass variation (expressed in percent) as a function of temperature

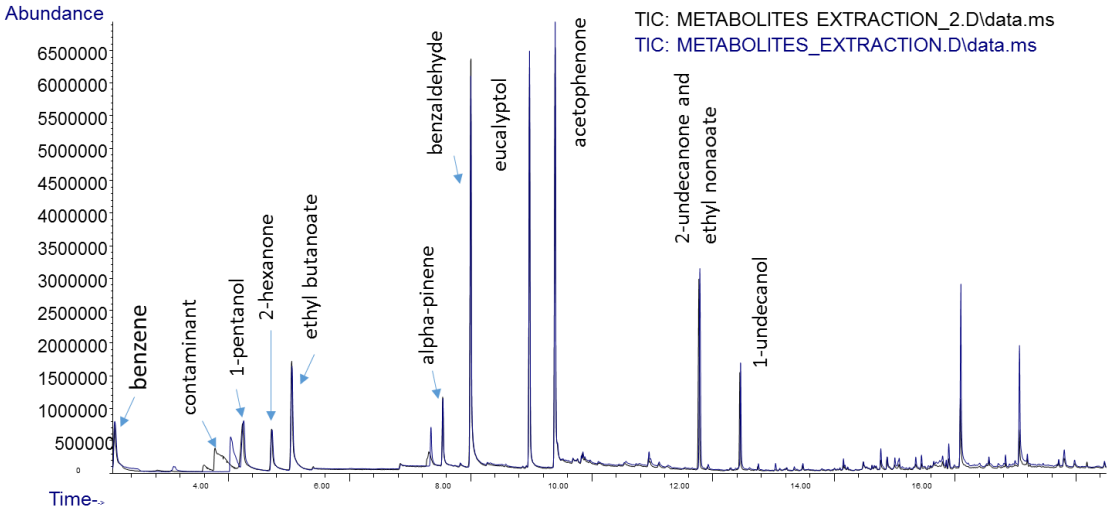

Figure S3: Overlapped chromatograms of two consecutive extractions of 11 probe compounds (namely, benzene, 1-pentanol, 2-hexanone, ethyl butanoate, α-pinene, benzaldehyde, eucalyptol, acetophenone, 2-undecanone, ethyl nonanoate, 1-undecanol) from aqueous media using PTFE AF/HLB coating

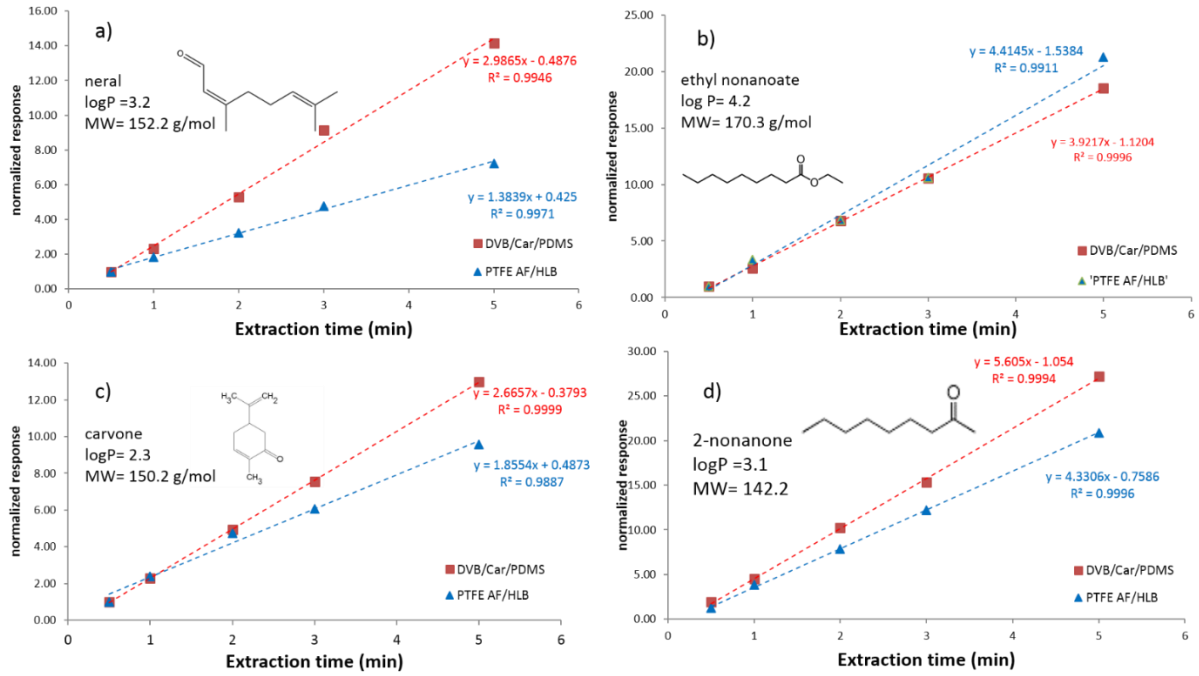

Figure S4: Comparison of uptake rates on PTFE AF/HLB and DVB/Car/PDMS for a) neral, b) ethyl nonanoate, c) 2-nonanone, d) 2-nonanone (representing analytes with low polarities and high molecular weights). Extractions were performed at 30ºC and 600 rpm in direct immersion mode

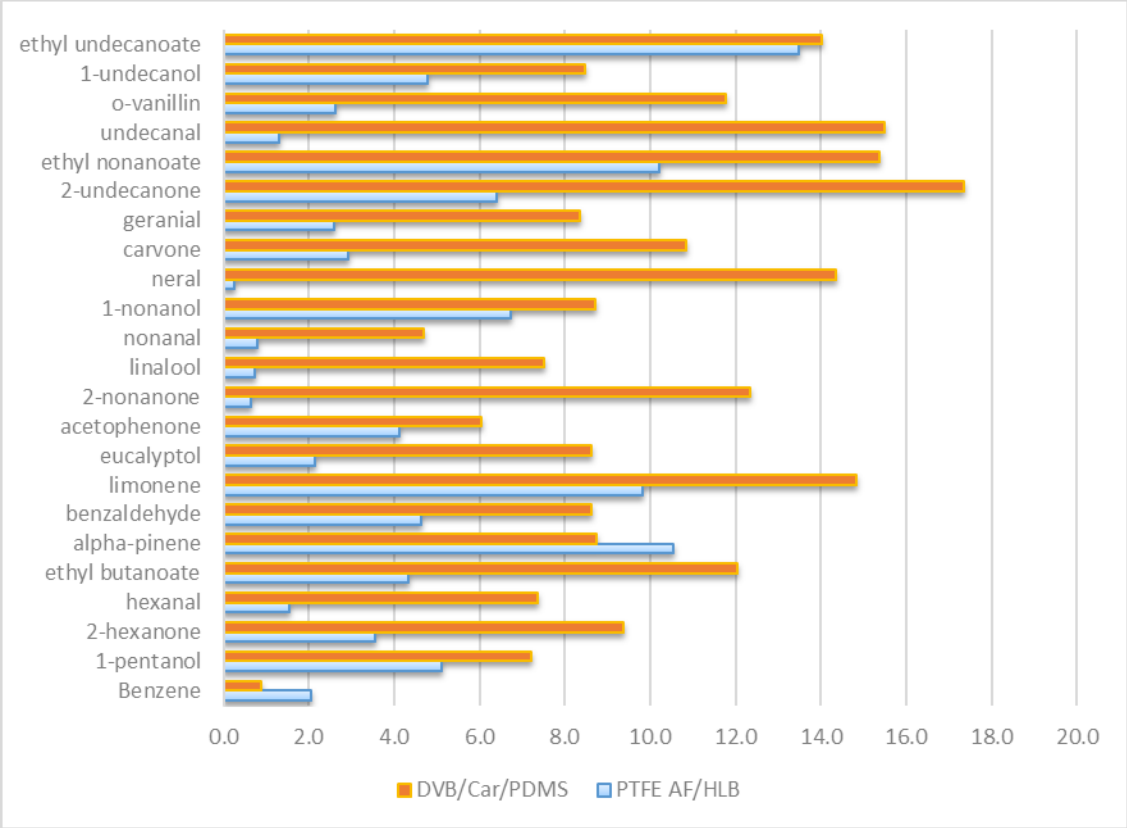

Figure S5: Relative standard deviations (RSD% n=3) obtained by DVB/Car/PDMS and PTFE AF/HLB for extraction of the probed GC-amenable analytes for 30 minutes and 600 rpm and 30°C in direct immersion mode.

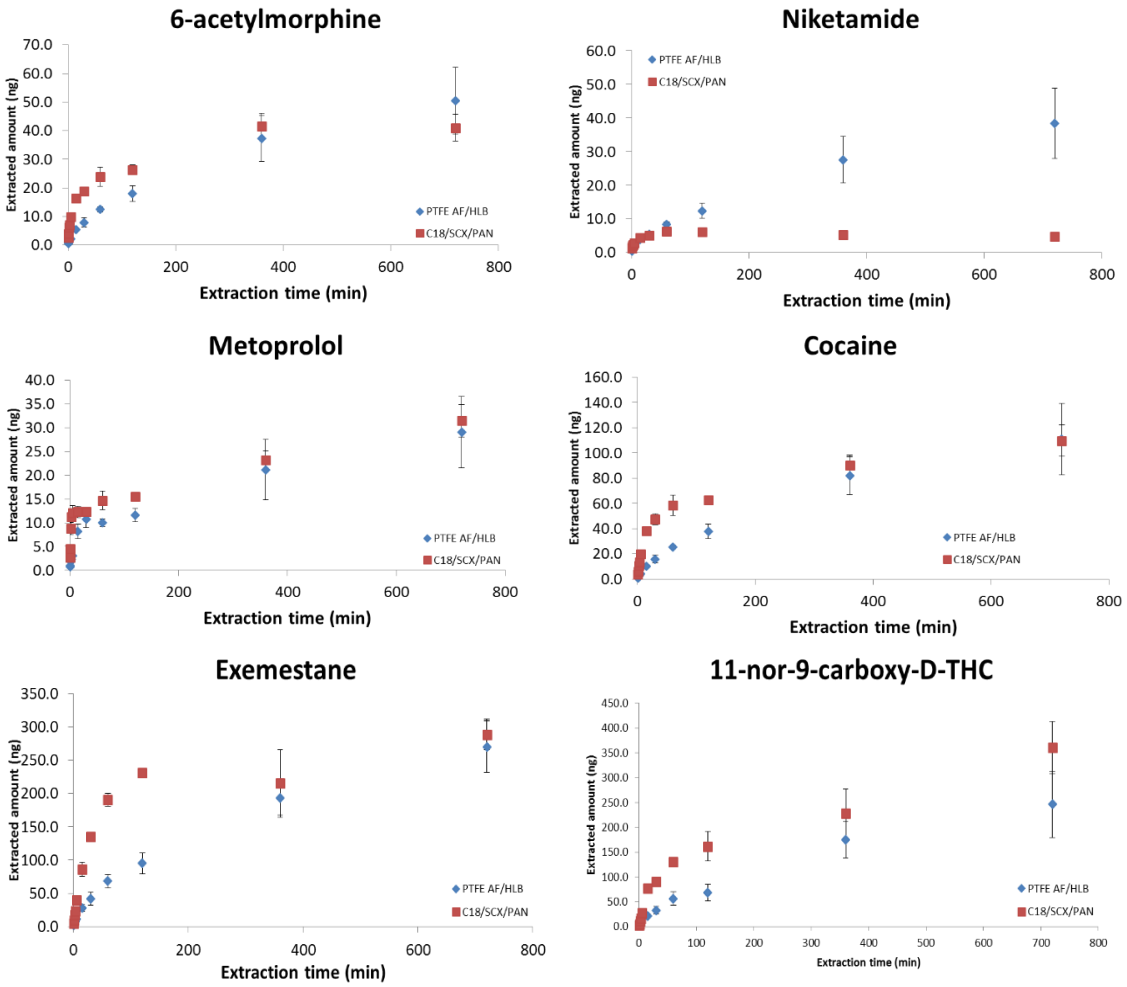

Figure S6: Extraction time profiles of representative compounds obtained by PTFE AF/HLB and C18/SCX/PAN coatings

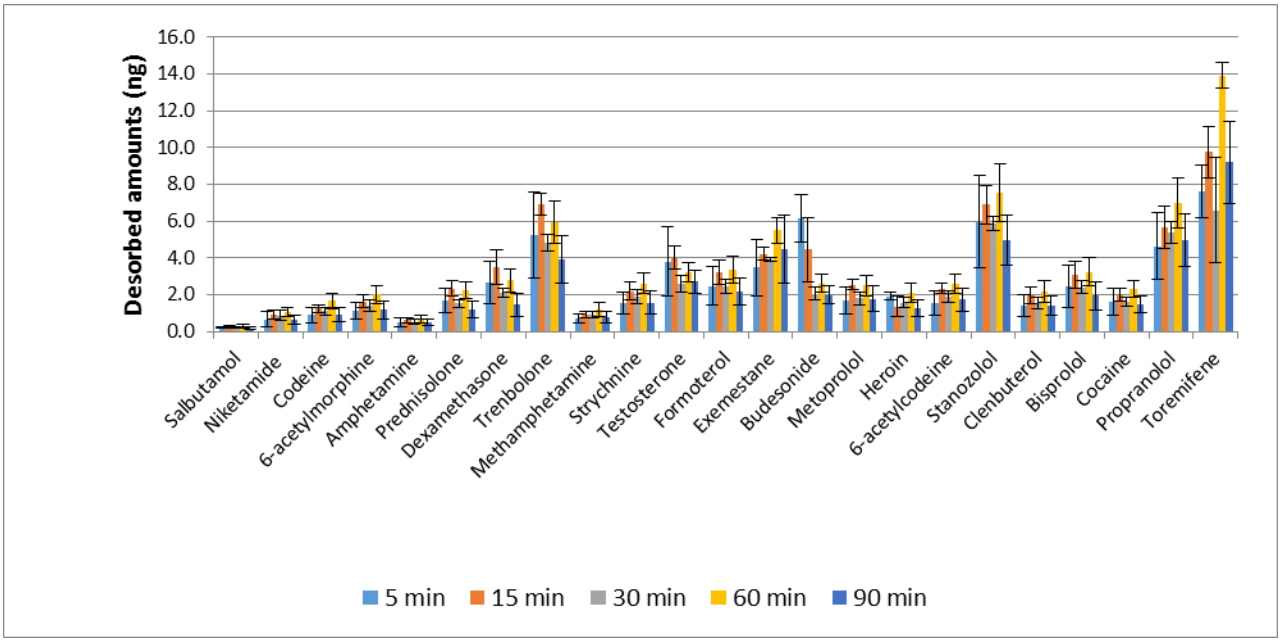

Figure S7: Desorption time profiles obtained by desorbing a PTFE AF/HLB coating in ACN/MeOH/H_2O (40/40/20, v/v/v) with 0.1% (v/v) FA at different desorption times; 5, 15,30,60,90 min.

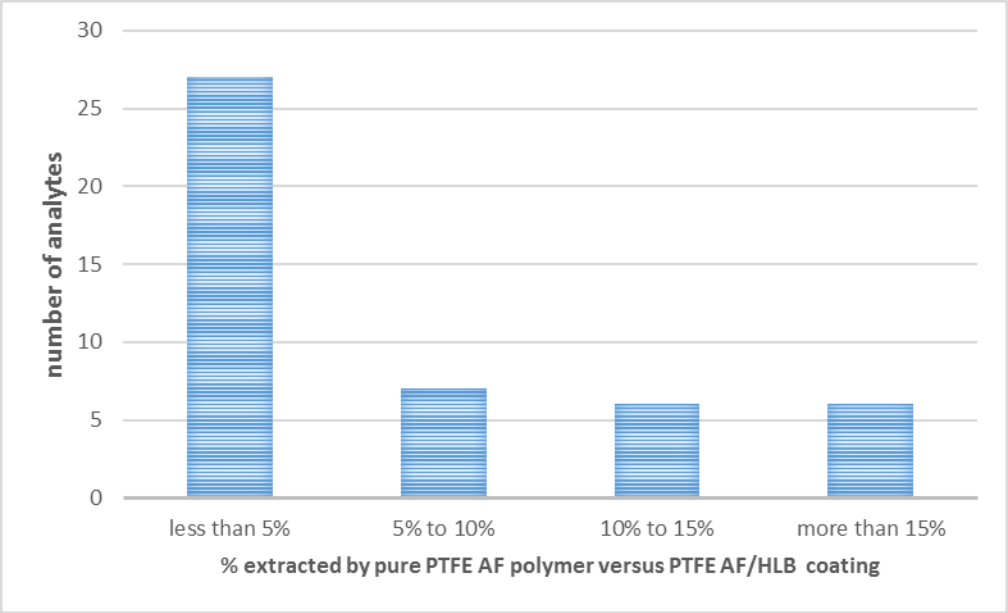

Figure S8. Percentage of the amount extracted by pure PTFE AF as a function of total amounts extracted by PTFE AF/HLB

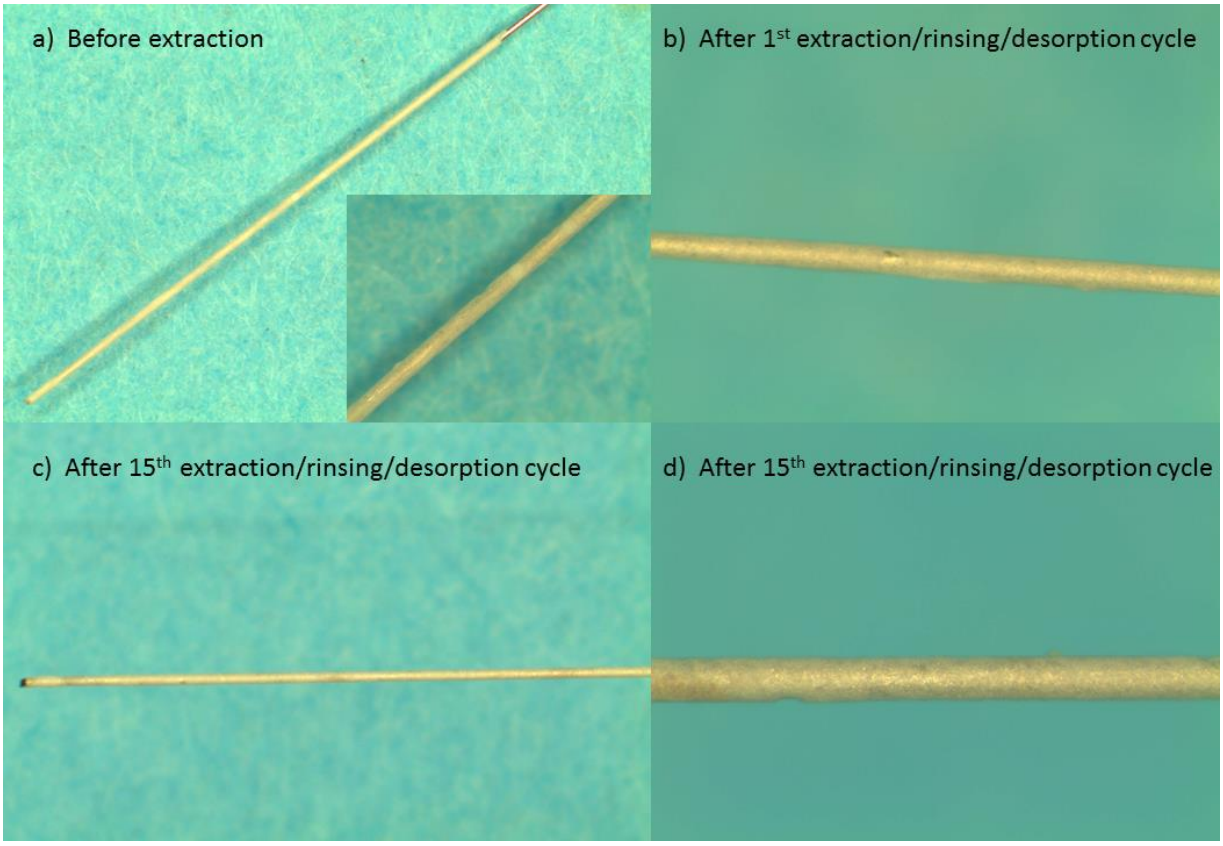

Figure S9. Microscopic images of the PTFE AF/HLB coating a) before extraction, b) after 30 min extraction in whole blood and consecutive rinsing and desorption, and c) and d) after 15 consecutive cycles of extraction, rinsing, and desorption

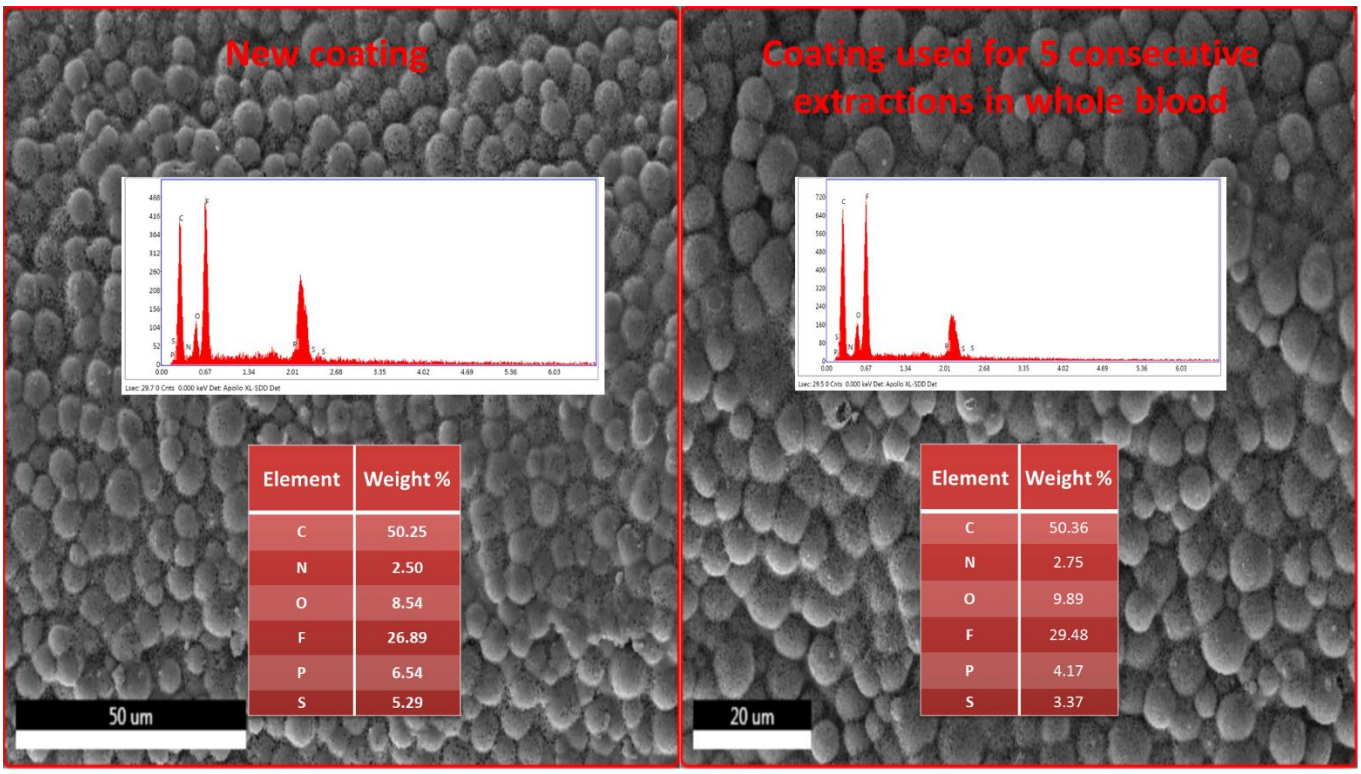

Figure S10. SEM images and EDX analysis for a new PTFE AF/HLB coating (right) and a PTFE AF/HLB coating used for 5 consecutive extraction in whole blood (left).

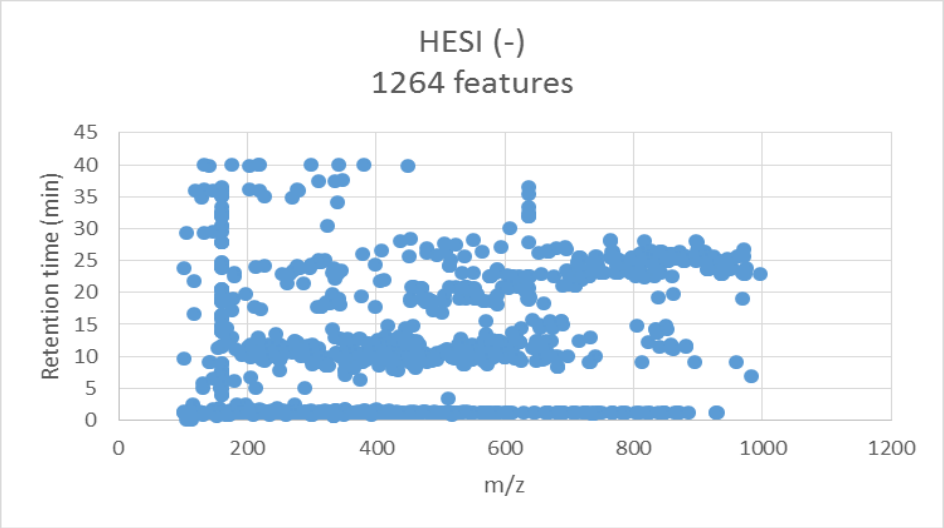

Figure S11. Ion map of apple extract performed by PTFE AF/HLB coatings in HESI (-) mode by using LC-HRMS

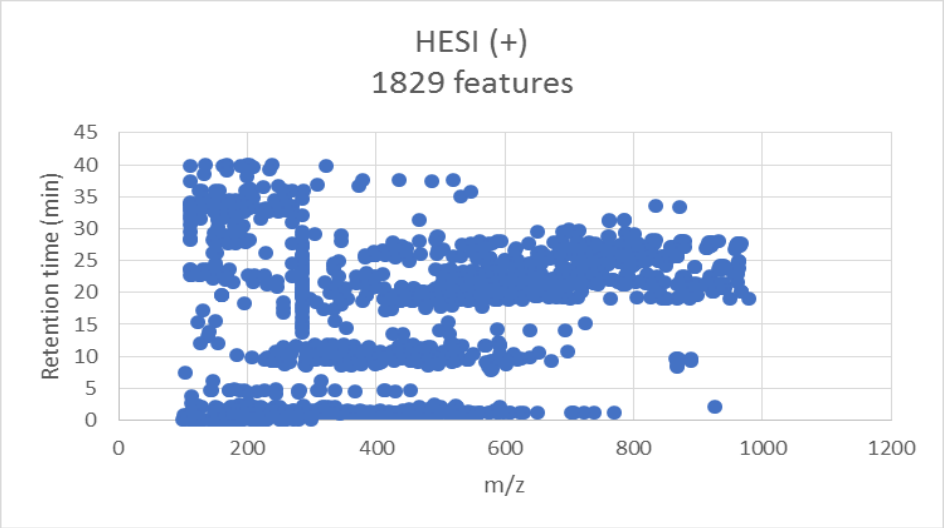

Figure S12. Ion map of apple extract performed by PTFE AF/HLB coatings in HESI (+) mode by using LC-HRMS

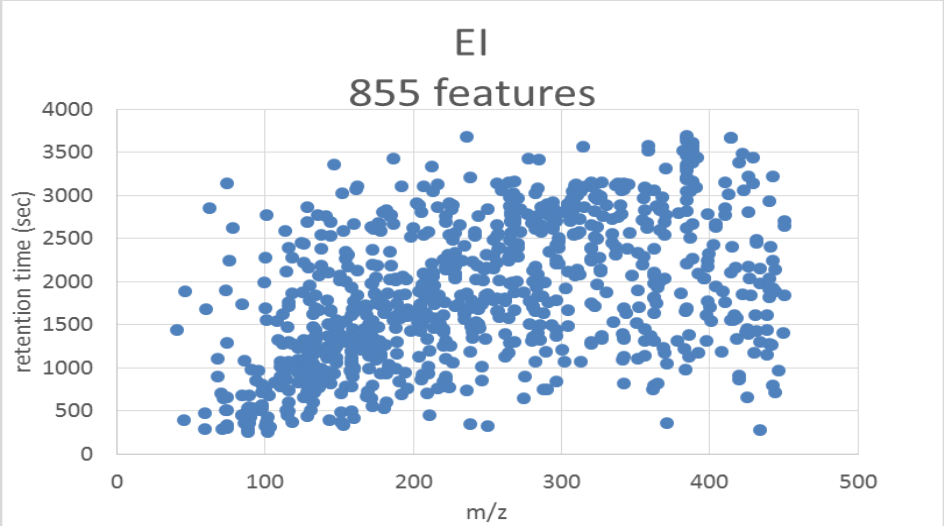

Figure S13. Ion map of apple extract performed by PTFE AF/HLB coatings in EI mode by using GC-ToF/MS

Table S1. Targeted analytes and selected internal standards used for this work

Compound	Supplier	Family	Formula[a]	Molecular Mass (Da)[a]	Log P[b]	Exact Mass m/z
GC-amenable compounds						
Benzene	Sigma Aldrich	Aromatic	C_6H_6	78.1	2.0	-
1-pentanol	Sigma Aldrich	Aliphatic alcohol	$C_5H_{12}O$	84.1	1.3	-
2-hexanone	Sigma Aldrich	Aliphatic ketone	$C_6H_{12}O$	100.2	1.4	-
Hexanal	Sigma Aldrich	Aliphatic aldehyde	$C_6H_{12}O$	100.2	1.9	-
Ethyl butanoate	Sigma Aldrich	Aliphatic ester	$C_6H_{12}O_2$	116.2	1.8	-
α-pinene	Sigma Aldrich	Terpene	$C_{10}H_{16}$	136.2	4.4	-
Benzaldehyde	Sigma Aldrich	Aromatic aldehyde	$C_6H_{12}O_2$	106.1	1.6	-
Limonene	Sigma Aldrich	Terpene	$C_{10}H_{16}$	136.2	4.4	-
Eucalyptol	Sigma Aldrich	Terpene	$C_6H_{12}O_2$	154.3	2.82	-
Acetophenone	Sigma Aldrich	Aromatic ketone	C_8H_8O	120.1	1.66	-
2-nonanone	Sigma Aldrich	Aliphatic ketone	$C_9H_{18}O$	142.2	3.03	-
Linalool	Sigma Aldrich	Terpene	$C_{10}H_{18}O$	154.2	3.28	-
Nonanal	Sigma Aldrich	Aliphatic aldehyde	$C_9H_{18}O$	142.2	3.6	-
2-nonanol	Sigma Aldrich	Aliphatic alcohol	$C_9H_{18}O$	144.3	3.4	-
Citral	Sigma Aldrich	Terpene	$C_9H_{18}O$	152.2	3.2	-
Carvone	Sigma Aldrich	Terpene	$C_9H_{18}O$	150.2	2.3	-
Geranial	Sigma Aldrich	Terpene	$C_9H_{18}O$	152.2	3.2	-
2-undecanone	Sigma Aldrich	Aliphatic ketone	$C_{11}H_{22}O$	170.3	4.1	-

Ethyl nonanoate	Sigma Aldrich	Aliphatic ester	$C_{11}H_{22}O_2$	186.3	4.4	-
Undecanal	Sigma Aldrich	Aliphatic aldehyde	$C_{11}H_{22}O$	170.3	4.6	-
1-undecanol	Sigma Aldrich	Aliphatic alcohol	$C_{11}H_{24}O$	172.3	4.5	-
Ethyl undecanoate	Sigma Aldrich	Aliphatic ester	$C_{13}H_{26}O_2$	214.3	5.5	-
LC-amenable compounds						
(±)11-Nor-9-carboxy-Δ^9-THC	Cerilliant	Cannabinoids	$C_{21}H_{28}O_4$	344.5	5.14	345.2063
6-Acetylcodeine	Cerilliant	Narcotics	$C_{20}H_{23}NO_4$	341.4	2.08	342.1703
6-Acetylmorphine	Cerilliant	Narcotics	$C_{19}H_{21}NO_4$	327.4	0.42	328.1547
(±) Amphetamine	Cerilliant	Stimulant	$C_9H_{13}N$	135.1	1.76	136.1123
Bisoprolol Hemifumarate	Sigma	Beta blocker	$C_{18}H_{31}NO_4$	325.2	1.87	326.2329
Budesonide	Sigma	Glucocorticosteroids	$C_{25}H_{34}O_6$	430.2	2.18	431.2433
Clenbuterol HCl	Sigma	Other anabolic	$C_{12}H_{18}Cl_2N_2O$[c]	277.2	2.33	277.0872
Cocaine	Cerilliant	Stimulant	$C_{17}H_{21}NO_4$	303.1	2.30	304.1547
Codeine	Cerilliant	Narcotics	$C_{18}H_{21}NO_3$	299.4	1.19	300.1598
Dexamethasone	Sigma	Glucocorticosteroids	$C_{22}H_{29}FO_5$	392.2	1.83	393.2076
Exemestane	Sigma	Hormones	$C_{20}H_{24}O_2$	296.2	3.70	297.1858
Formoterol fumarate dihydrate	Sigma	Beta agonist	$C_{19}H_{24}N_2O_4$	344.2	2.20	345.1812
Heroin	Cerilliant	Narcotics	$C_{21}H_{23}NO_5$	369.2	1.58	370.1646
(±) Methamphetamine	Cerilliant	Stimulant	$C_{10}H_{15}N$	149.1	2.07	150.1280
(±)Metoprolol-(+) tartarate salt	Sigma	Beta blocker	$C_{15}H_{25}NO_3$[c]	267.2	1.60	268.1911
Nikethamide	Sigma-Aldrich	Stimulant	$C_{10}H_{14}N_2O$	178.2	0.33	179.1182
Prednisolone	Sigma	Glucocorticosteroids	$C_{21}H_{28}O_5$	360.2	1.62	361.2014
(±)Propranolol HCl	Sigma	Beta blocker	$C_{16}H_{21}NO_2$	259.3	3.48	260.1649
Salbutamol	Sigma-Aldrich	Beta agonist	$C_{13}H_{21}NO_3$	239.2	0.64	240.1598
Stanozolol	Cerilliant	Steroids	$C_{21}H_{32}N_2O$	328.3	3.81	329.2591

(-) Strychnine	Fluka	Stimulant	$C_{21}H_{22}N_2O_2$	334.2	1.93	335.1758
Testosterone	Sigma	Steroids	$C_{19}H_{28}O_2$	288.2	3.32	289.2166
17α-Trenbolone	Cerilliant	Steroids	$C_{18}H_{22}O_2$	270.2	2.27	271.1696
Internal standards						
(±)11-nor-9-carboxy-Δ^9-THC-d3	Cerilliant		$C_{21}H_{25}D_3O_4$	347.46	-	
6-Acetylmorpine-d3	Cerilliant		$C_{19}D_3H_{18}NO_4$	330.4	-	331.1740
Cocaine-d3	Cerilliant		$C_{17}D_3H_{18}NO_4$	306.1	-	307.1737
Codeine-d3	Cerilliant		$C_{18}D_3H_{18}NO_3$	302.4	-	303.1781
Cortisol-d4	Cerilliant		$C_{21}D_4H_{26}O_5$	362.5	-	367.2424
Salbutamol-d3	CDN Isotopes		$C_{13}D_3H_{18}NO_3$	242.2	-	243.1783
Testosterone d3	Fluka		$C_{19}D_3H_{25}O_2$	291.2	-	292.2355

Table S2. Figures of merit achieved for the PTFE AF/HLB coating (extraction time: 30 min, desorption time: 30 min)

Analytes	MRPL	LOQ	R^2	Internal Standard	Intra-fiber reproducibility	Inter-fiber reproducibility
		ng mL^{-1}			RSD % (N:3)	RSD % (N:3)
6-acetylmorphine	50	1.0	0.998	6-acetylmorphine_d3	5	7
Nikethamide	100	1.0	0.998	6-acetylmorphine_d3	11	2
Salbutamol	100	1.0	0.992	Testosterone_d3	15	3
Codeine	50	1.0	0.999	6-acetylmorphine_d3	1	14
Heroin	50	0.5	0.999	Cocaine_d3	12	2
Metoprolol	100	5.0	0.997	Testosterone_d3	8	9
Prednisolone	30	5.0	0.999	Cortisol_d4	10	6
Amphetamine	100	0.5	0.998	Codeine_d3	7	6
Dexamethasone	30	1.0	0.999	Cortisol_d4	11	23
Bisoprolol	100	1.0	0.999	Cocaine_d3	6	10
Strychnine	100	1.0	0.999	Cocaine_d3	9	2
Methamphetamine	100	5.0	0.999	Codeine_d3	12	5
6-acetylcodeine	50	5.0	0.999	Cocaine_d3	14	22
Formoterol	100	1.0	0.999	Cocaine_d3	6	7
Trenbolone	5	5.0	0.999	Cortisol_d4	8	2
Cocaine	100	1.0	0.998	Cocaine_d3	14	11
Budesonide	30	0.5	0.999	Testosterone_d3	7	0
Clenbuterol	0.2	1.0	0.996	6-acetylmorphine_d3	25	13
Testosterone	5	1.0	0.999	Testosterone_d3	5	5
Propranolol	100	5.0	0.999	Cocaine_d3	13	2
Exemestane	20	1.0	0.999	Testosterone_d3	12	3
Stanozolol	2	5.0	0.998	11-or-9-carboxy-D-THC_d3	23	5
11-nor-9-carboxy-D-THC	2	0.5	0.998	11-or-9-carboxy-D-THC_d3	31	3

Table S3 Absolute matrix effect obtained from analysis of whole blood spiked at two different spike levels (10 and 100 ng mL^{-1}).

	% Absolute matrix effect*			
	After first extraction		After 5 extractions	
	10 ng mL^{-1}	100 ng mL^{-1}	10 ng mL^{-1}	100 ng mL^{-1}
6-Acetylcodeine	109 (± 24)	90 (± 14)	115 (± 15)	99 (± 2)
6-Acetylmorphine	111 (± 16)	97 (± 4)	110 (± 14)	95 (± 2)
Amphetamine	99 (± 15)	106 (± 10)	95 (± 13)	107 (± 5)
Bisoprolol	110 (± 20)	99 (± 5)	118 (± 3)	108 (± 5)
Budesonide	105 (± 10)	110 (± 1)	106 (± 7)	103 (± 2)
Clenbuterol	115 (± 28)	116 (± 19)	93 (± 8)	104 (± 5)
Cocaine	103 (± 15)	106 (± 2)	103 (± 9)	106 (± 1)
Codeine	106 (± 16)	103 (± 2)	106 (± 8)	104 (± 2)
Dexamethasone	110 (± 11)	113 (± 4)	107 (± 5)	115 (± 3)
Exemestane	101 (± 14)	105 (± 6)	104 (± 11)	105 (± 4)
Formoterol	116 (± 20)	89 (± 7)	116 (± 12)	90 (± 1)
Heroin	102 (± 16)	97 (± 15)	109 (± 12)	94 (± 6)
Methamphetamine	109 (± 12)	110 (± 10)	104 (± 11)	104 (± 5)
Nikethamide	106 (± 11)	91 (± 1)	103 (± 8)	93 (± 3)
Prednisolone	103 (± 14)	108 (± 6)	109 (± 10)	107 (± 3)
Propranolol	101 (± 15)	106 (± 3)	99 (± 11)	104 (± 1)
Salbutamol	113 (± 17)	99 (± 7)	130 (± 16)	100 (± 8)
Stanozolol	113 (± 17)	99 (± 7)	130 (± 16)	100 (± 8)
Strychnine	121 (± 22)	95 (± 0)	129 (± 9)	99 (± 3)
Testosterone	108 (± 20)	96 (± 4)	108 (± 17)	93 (± 4)
17α-Trenbolone	106 (± 13)	98 (± 1)	106 (± 9)	98 (± 3)

* Absolute matrix effect defined as % of spiked desorption solvent from SPME of blank matrix (10 or 100 ng mL -1) *vs*. spiked neat solvent (10 or 100 ng mL -1)).

Table S4 Student's t-test for independent samples performed on *fc* values obtained by PTFE AF/HLB and DVB/Car/PDMS coatings.

	t-score	p-level %
Benzene	0.87	43.42
1-pentanol	27.34	0.001
2-hexanone	36.75	0.0003
hexanal	80.93	0.00001
ethyl butanoate	26.05	0.00129
alpha-pinene	14.62	0.012
benzaldehyde	11.36	0.034
limonene	3.33	2.91
eucalyptol	91.39	0.00001
acetophenone	1.6	18.4
2-nonanone	2.69	5.45
linalool	26.66	0.0012
nonanal	0.61	57.61
1-nonanol	0.23	83.25
neral	12.62	0.0227
carvone	9.83	0.06
geranial	3.95	1.68
2-undecanone	1.22	28.95
ethyl nonanoate	1.6	18.38
undecanal	0.53	62.383
o-vanillin	4.36	1.2
1-undecanol	1.8	14.67
ethyl undecanoate	4.99	0.75