Solid phase microextraction devices prepared on plastic support and their evaluation as potential single use samplers in bioanalytical applications

Nathaly Reyes-Garcés [a], Barbara Bojko [a], Dietmar Hein, Janusz Pawliszyn [a,*]

[a]*Department of Chemistry, University of Waterloo, Ontario, Canada N2L 3G1*

[b]*PAS Technology, Magdala, Germany*

* Corresponding author: Tel.: +1 519 888 4641; fax: +1 519 746 0435.

E-mail address: Janusz@uwaterloo.ca (J. Pawliszyn).

Supporting Information

Summary

This file contains additional information regarding procedures and data described in the main manuscript. The supporting information herein presented includes the following points: materials and supplies, details about LC-MS/MS conditions, selective reaction monitoring transitions used to quantify and qualify each model compound, inter-device reproducibility results, blank of the new proposed devices, results corresponding to matrix effects estimated using the presented SPME devices, a comparison of absolute recoveries obtained with and without applying an additional polyacrylonitrile layer, an evalution of different rinsing strategies, microscope and SEM images taken after exposing the coated devices to whole blood, evaluation of absolute matrix effect after blood extraction, microscope pictures of thin-film devices prepared on PBT support (side view), transition ratios calculated from the standard and tested matrices (urine, plasma and blood), and average absolute recoveries obtained when using HLB thin-films prepared on PBT.

Materials and supplies

A 250 mL flask-type sprayer, formic acid, PAN, potassium chloride, sodium chloride, potassium phosphate monobasic, and sodium phosphate dibasic were obtained from Sigma-Aldrich (Oakville, ON, Canada). Oasis hydrophilic-lipophilic balance 30 µm sorbent particles (HLB) were obtained from Waters (Milford, MA, USA). Discovery silica-based C18 5 µm particles were gently provided by Supelco Sigma-Aldrich (Bellefonte, PA, USA). N,N-dimethyl formamide (DMF) was purchased from Caledon Labs (Georgetown, ON, Canada). PBT rounded pieces (1.7 mm diameter) were obtained from Professional Analytical System (PAS) Technologies (Magdala, Germany), and PBT film (300 mm width, 2 m length, 0.5 mm thickness) was purchased from Goodfellow (London, UK). Polypropylene Nunc U96 deep well plates were obtained from VWR international (Mississauga, ON, Canada) and LC-MS grade solvents (acetonitrile, methanol, and water) were purchased from Fischer Scientific.

A phosphate-buffered saline solution (PBS) (pH 7.4) was prepared by adding 8.0 g of sodium chloride, 0.2 g of potassium chloride, 0.2 g of potassium phosphate and 1.44 g of sodium phosphate to 1 L of nanopure water. Pooled human plasma and whole blood from healthy donors in potassium (K_2) ethylenediaminetetraacetic acid (EDTA) were purchased from Lampire Biological Laboratories (Pipersville, PA, USA). Urine samples were collected from two healthy volunteers (one female and one male). Collection of urine from healthy volunteers for this particular study was under the approval of the Office of Research Ethical Board of University of Waterloo.

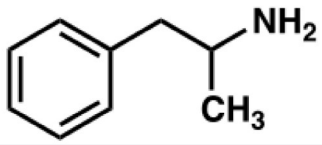
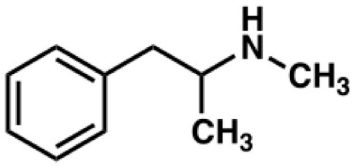
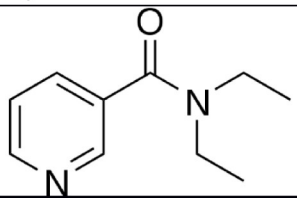
LC-MS/MS conditions

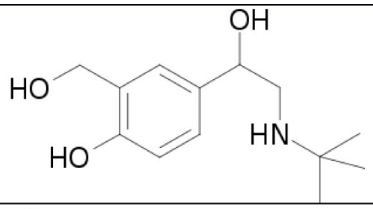
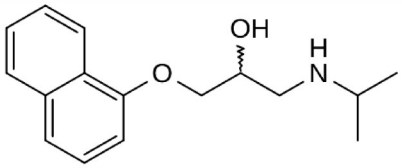
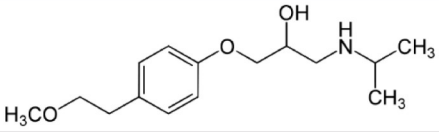
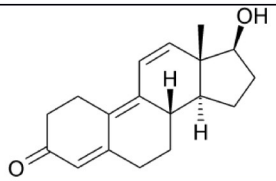
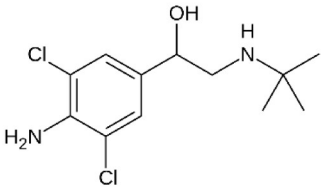
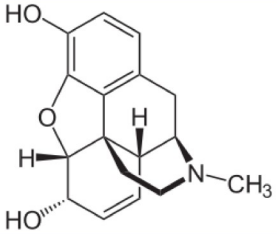
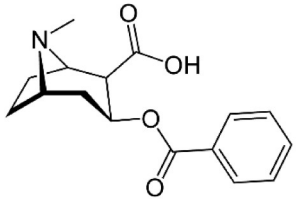
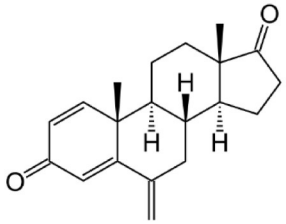
All the extracts were run using an LC-MS/MS system comprised by an Accela autosampler, an Accela pump and a triple quadrupole mass spectrometer TSQ vantage with a heated electrospray ionization source operating in positive mode (Thermo Scientific, San Jose, USA). For chromatographic separation, a pentafluorophenyl core shell column (1.7 µm, 2.1 mm × 10 mm) with guard (PFP security guard ultracartridge) was employed (Phenomenex, Torrance, CA, USA). A ternary mobile phase system consisting of 0.1 % formic acid (A), acetonitrile with 0.1 % formic acid (B) and methanol with 0.1 % formic acid (C) was used for LC separation. Gradient elution conditions were set as follows: A, B and C were hold at 90, 5 and 5 %, respectively, for 0.5 min, B and C were linearly increased to 50 % in 6.5 min, then C was increased to 75 % and B decreased to 25% in 5 min and held for 3.5 min. Finally, the column was kept at the initial gradient composition for 2 min. The column temperature was maintained at 35 ˚C, the total run time was 17.5 min, and the column flow was set at 0.3 mL/min. Samples were stored in the autosampler at 5 ˚C and the injection volume was 10 µL. MS analysis was carried out using selective reaction monitoring (SRM) mode (see Table S1) and conditions were optimized by doing direct infusion of the standards. Other parameters were the following: spray voltage = 1300 V, vaporizer temperature = 275◦C, sheath gas = 45 units, auxiliary gas = 30 and capillary temperature = 280◦C.

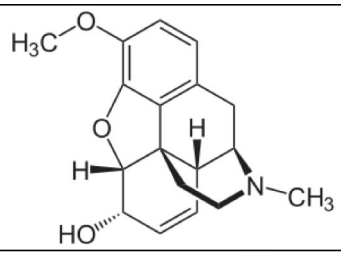
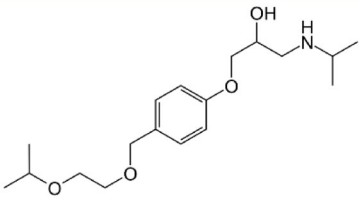
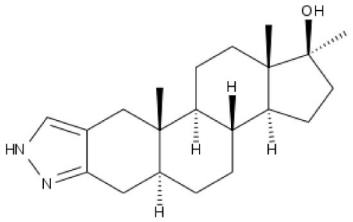
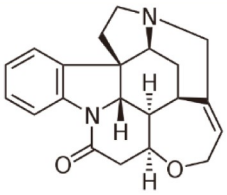
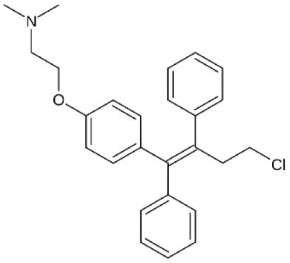
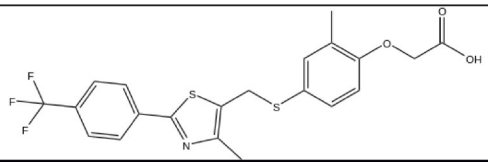
Table S1. List of support materials commonly used to manufacture SPME devices

Material (geometry)	SPME coating application	Advantages	Disadvantages
Fused silica (fibers)	GC	Inertness, thermal stability, availability and low cost	It can easily break, especially in the point where the coating contacts the fibre plunger.
StableFlex (fused silica coated with a thermally stable polymer) (fibers)	GC	It is more robust than bare fused silica. Provides more stable attachment for some adsorbent coatings.	Thermally stable at maximum 320 °C. Depending on extraction conditions, artefact peaks could appear.
Non-ferrous alloy (nitinol) (fibers)	GC and LC	Flexibility, tensile strength, shape memory properties, thermal stability (450 °C) and inertness.	High cost
Stainless steel (thin-films)	LC	Accessibility and robustness.	Presence of iron can lead to a lower inertness compared to nitinol. Limited flexibility.

Table S2. Target analytes and physical chemical properties

Compound	Structure	Log P	pKa	Charge at physiological pH[d]
Amphetamine[a]		1.76	10.01	1
Methamphetamine[a]		2.07	10.21	1
Nikethamide[b]		0.33	3.61	0

Salbutamol[b]		0.64	9.4 and 10.12	1
Propranolol[b]		3.48	9.67 and 14.09	1
Metoprolol[b]		1.88	9.67 and 14.09	1
17-α-trenbolone[a]		2.27	-	0
Clenbuterol[b]		2.61	9.63 and 14.06	1
Morphine[a]		0.89	9.12 and 10.26	1
Benzoylecgonine[a]		2.71	3.15 and 9.54	0
Exemestane[b]		3.11	-	0

Codeine[a]		1.19	9.19 and 13.78	1
Bisoprolol[b]		1.87	9.67 and 14.09	1
Stanozolol[a]		4.42	2.86	0
Strychnine[b]		1.93	9.27	1
Toremifene[c]		6.56	8.76	1
GW501516[c]		6.46	2.14 and 3.51	-1

[a]These standards and codeine-d3, oxycodone-d3, cannabidiol-d3, methadone-d3, (±)11-nor-9-carboxy-Δ9-THC-d3 (THCCOOH-d3)were obtained from Cerilliant Corporation (Round Rock, TX, USA).

[b]These standards and testosterone-d_3 were obtained from Sigma-Aldrich (Oakville, ON, Canada). Salbutamol-d_3 was purchased from CDN Isotopes (Pointe Claire, Quebec, Canada).

[c]Standards were obtained from Toronto Research Chemical (Toronto, ON, Canada).

[d]More than 90 % of each analyte is present at that particular ionized form.

Table S3. Transitions monitored for each model compound in positive ionization mode (quantifier transition underlined).

Compound	Retention time	Parent ion (m/z)	Product ion (m/z)	Collision energy	Windows, min		S-Lenses
					Start time	End time	
Amphetamine	4.73	136.099	65.138	36	3.8	5.8	36
			<u>91.114</u>	17			
Methamphetamine	5.54	150.112	<u>91.12</u>	19	4.6	6.6	45
			119.139	9			
Nikethamide	2.84	179.1	80.127	29	1.8	3.8	76
			<u>108.102</u>	18			
Salbutamol	2.37	240.143	<u>148.103</u>	18	1.3	3.3	59
			166.116	12			
Salbutamol-d_3	2.37	243.16	<u>151.123</u>	18	1.3	3.3	64
			169.138	12			
Propranolol	9.51	260.123	<u>116.138</u>	17	8.5	11.0	89
			183.116	17			
Metoprolol	6.31	268.14	77.105	50	5.4	7.4	94
			<u>116.146</u>	18			
Trenbolone	5.32	271.133	<u>165.106</u>	56	4.2	6.2	97
			199.17	24			
Clenbuterol	6.89	277.068	132.1	30	6.0	8.0	70
			<u>203.049</u>	15			
Morphine	1.96	286.119	<u>152.092</u>	61	1.0	3.0	110
			165.101	40			
Benzoylecgonine	4.62	290.133	77.141	47	3.5	6.5	93
			<u>168.164</u>	18			
Testosterone-d_3	5.72	292.248	97.135	22	4.5	6.7	93
			109.137	25			
Exemestane	6.04	297.173	91.128	39	5.0	7.0	72
			<u>121.118</u>	19			
Codeine	3.84	300.105	<u>152.092</u>	64	2.8	4.8	104
			165.102	42			
Codeine-d_3	3.84	303.149	165.096	41	2.8	4.8	104
			215.138	25			
Methadone-d_3	12.96	313.214	105.091	29	12.0	17.0	87
			268.224	13			
Bisoprolol	7.38	326.16	74.126	27	6.4	8.4	102
			<u>116.135</u>	17			
Stanozolol	6.66	329.229	<u>81.108</u>	44	5.5	7.5	130

			95.115	38			
Strychnine	5.53	335.155	156.126	45	4.6	6.6	136
			184.129	36			
(±)11-nor-9-carboxy-Δ9-THC-d_3	6.93	348.162	196.204	27	6.0	8.0	95
			302.282	18			
Toremifene	12.39	406.21	70.157	36	11.0	14.0	108
			72.167	24			
GW501516	7.52	454.091	188.079	46	6.5	8.5	108
			257.068	29			
Cannabidiol-d_3	7.17	318.146	123.041	33	6.0	8.0	82
			196.129	22			
Oxycodone-d_3	4.51	319.118	244.13	28	3.5	5.5	82
			259.155	25			

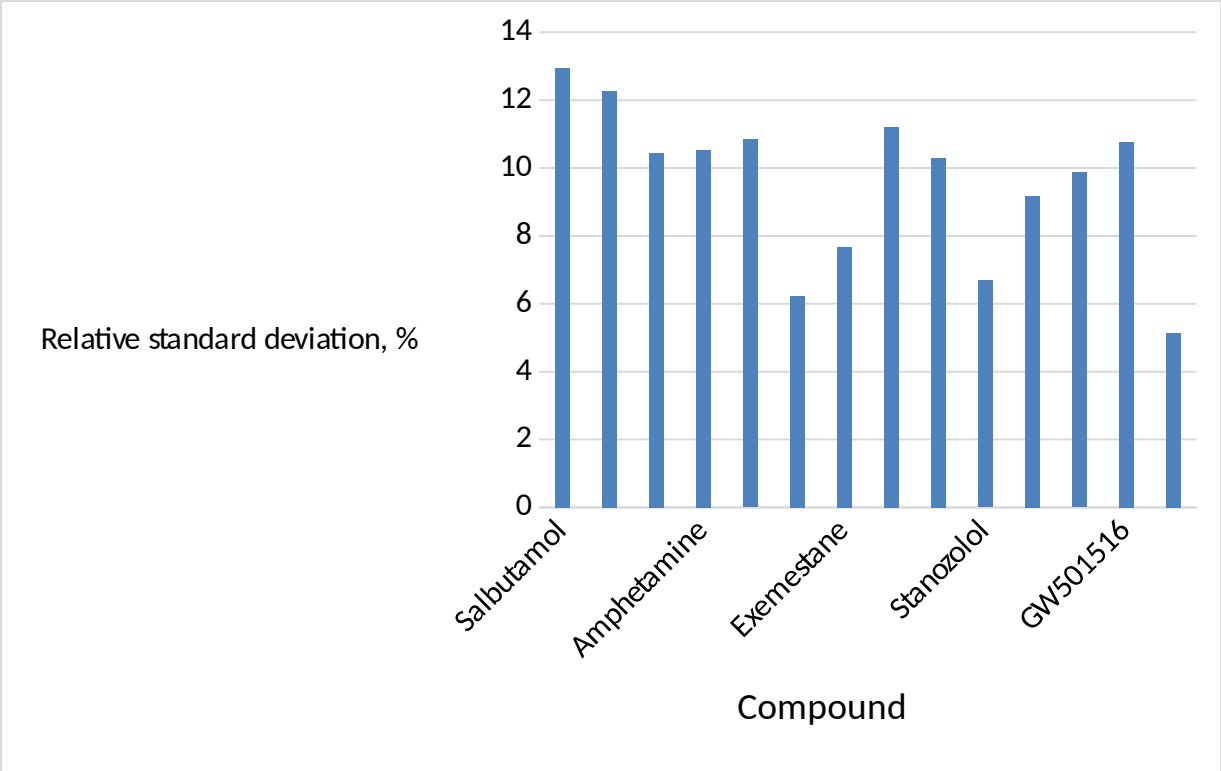


Figure S1. Inter-device relative standard deviation values (%) obtained from coated rounded PBT pins (n=20). Extractions were performed from PBS spiked at 50 ng mL^{-1}. The extraction time was 45 min.

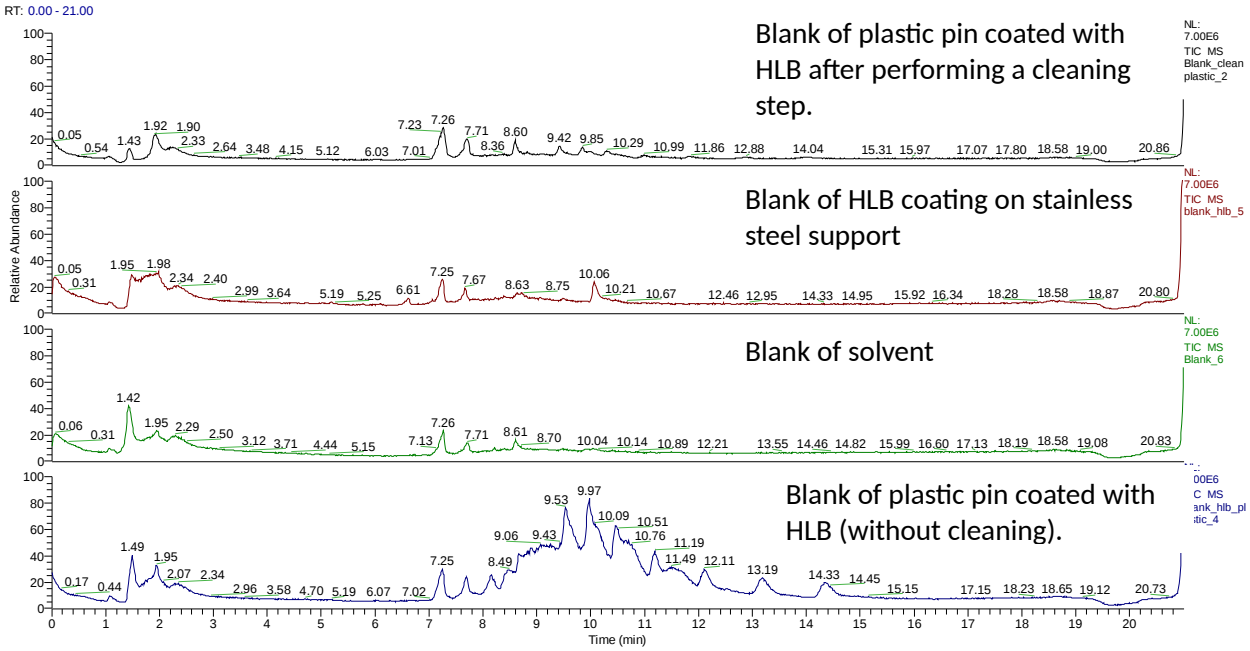


Figure S2. Blanks run in positive full scan mode (100 – 1000 m/z) using TSQ vantage. A cleaning step was performed by exposing the rounded coated PBT devices to a mixture of organic solvents (2:1:1 v/v methanol:acetonitrile:isopropanol) for 60 min under vortex agitation conditions

Table S4. Evaluation of absolute matrix effects in blank solution coming from desorption of new plastic HLB devices, and in extracts of blank urine and plasma obtained with such plastic devices (n=6, extracts spiked at 50 ng mL^{-1} and analyzed in positive ionization mode).

Compound	Absolute matrix effects, %		
	Plastic devices blank extract	Plasma blank extract	Urine blank extract
Morphine	99	106	93
Salbutamol	100	107	112
Nikethamide	101	108	108
Codeine	99	107	88
Benzoylecgonine	96	110	107
Amphetamine	95	103	102
Methamphetamine	100	105	101
Trenbolone	97	103	81
Strychnine	94	94	88
Metoprolol	98	91	95
Exemestane	95	97	87
Clenbuterol	99	92	94
Stanozolol	94	92	93
Bisoprolol	99	89	79
GW501516	101	103	103
Propranolol	99	99	90
Toremifene	103	107	102

Table S5. Enrichment factors calculated in the different matrices evaluated.

Compound	Enrichment factors (Cextract/Csample)		
	Urine (RSD, %)	Plasma (RSD, %)	Blood (RSD, %)
Morphine	0.56 (6)	0.52 (6)	0.62 (4)
Salbutamol	0.65 (2)	0.21 (6)	0.33 (1)
Nikethamide	1.03 (3)	0.80 (5)	0.90 (9)
Codeine	0.90 (7)	0.71 (7)	0.85 (3)
Benzoylecgonine	0.59 (7)	0.51 (6)	0.42 (7)
Amphetamine	0.89 (9)	0.39 (7)	0.61 (1)
Methamphetamine	1.10 (9)	0.60 (8)	0.73 (3)
Strychnine	1.10 (5)	0.28 (12)	0.53 (4)
Exemestane	1.41 (3)	0.22 (9)	0.27 (20)
Trenbolone	1.34 (4)	0.32 (10)	0.47 (11)
Metoprolol	1.20 (4)	0.69 (13)	0.96 (4)
Stanozolol	1.59 (6)	0.02 (12)	0.06 (12)
Clenbuterol	1.33 (8)	0.52 (10)	0.86 (5)
Bisoprolol	1.12 (7)	0.75 (11)	0.97 (6)
GW501516	1.44 (7)	0.01 (16)	0.02 (10)
Propanolol	1.42 (6)	0.29 (7)	0.20 (10)
Toremifene	1.22 (12)	0.01 (14)	0.04 (12)

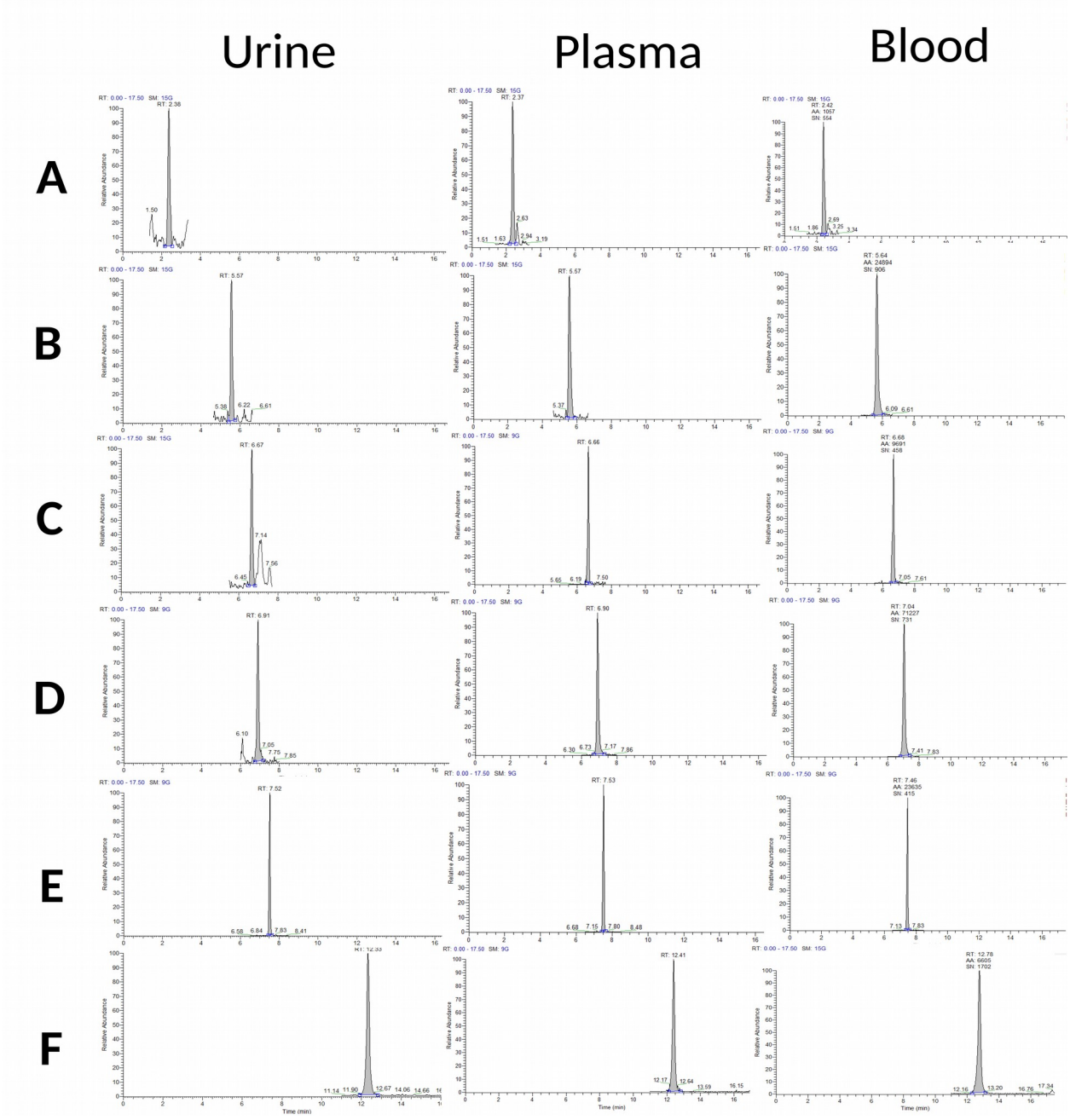


Figure S3. Representative SRM chromatograms corresponding to extracts obtained from urine, plasma and whole blood spiked at LOQ levels. Salbutamol (A), methamphethamine (B), stanozolol (C), clenbuterol (D), GW501516 (E), and toremifene (F).

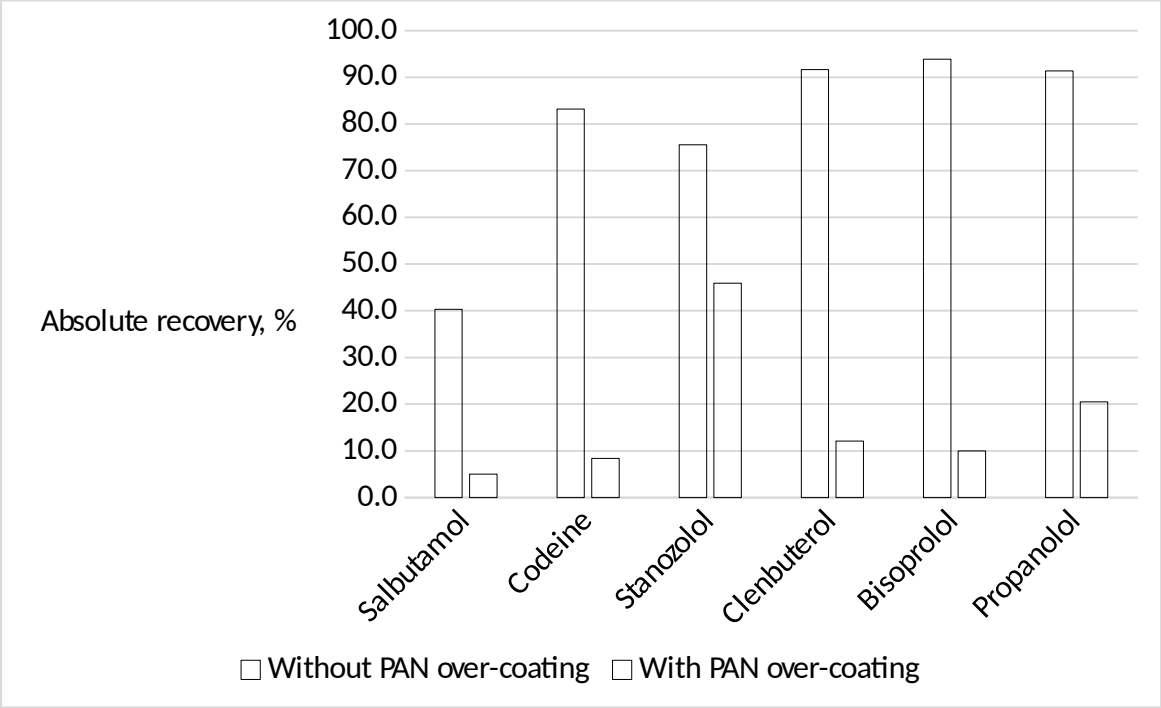


Figure S4. Comparison of absolute recoveries found using HLB thin films with and without PAN over-coating applied by dipping. Extractions were performed from PBS spiked at 70 ng mL^{-1}. The extraction time was 60 min.

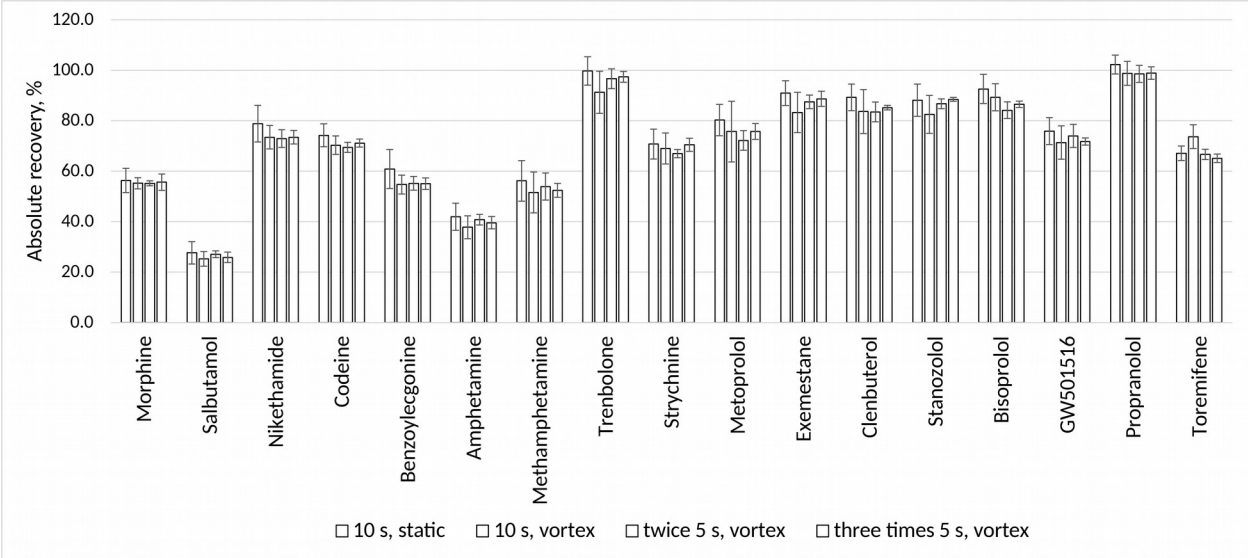


Figure S5. Evaluation of the effect of four different washing step approaches (10 s static, 10 s with vortex, two 5 s steps with vortex and three 5 s steps with vortex) on the final amount desorbed from rounded SPME-HLB-PBT devices (n = 4). Extractions were performed from PBS spiked at 50 ng mL^{-1} and mixed with 1 M buffer (9:1 ratio). The extraction time was set to 45 min.

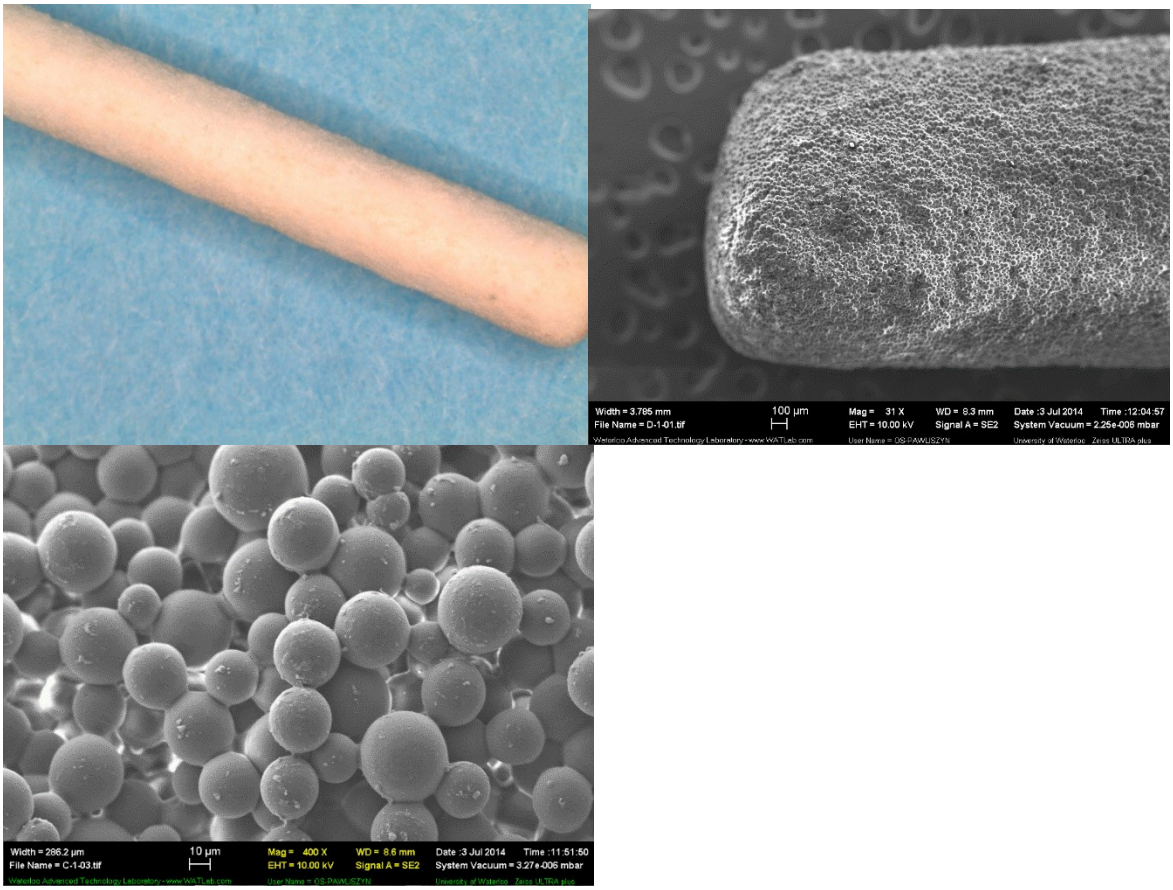


Figure S6. Microscope picture and SEM images (30x magnification) taken from SPME-HLB-PBT devices after being exposed for 90 min to whole blood.

Table S6. Absolute matrix effects assessed in whole blood

Compound	Ionization mode	Blank blood extract spiked at 50 ppb		Neat solvent, 50 ng mL^{-1}		Absolute matrix effects, %
		Average area counts	RSD, %	Average area counts	RSD, %	
Morphine	+	790613	6.8	746645	6.5	106
Salbutamol	+	1752803	6.4	1683275	7.4	104
Nikethamide	+	1770978	9.4	1698455	12.0	104
Codeine	+	901379	6.6	865793	6.5	104
Benzoylecgonine	+	2123832	5.5	2011989	5.7	106
Amphetamine	+	613793	7.8	597718	9.7	103
Methamphetamine	+	1472182	7.4	1403228	8.4	105
Strychnine	+	982709	4.9	892247	5.0	110
Exemestane	+	294528	3.8	284409	4.6	104
Trenbolone	+	294309	6.9	290659	7.0	101
Metoprolol	+	810327	7.8	807978	8.2	100
Stanozolol	+	2112271	4.9	2073968	5.3	102
Clenbuterol	+	2884970	6.2	2804007	7.6	103
Bisoprolol	+	4809840	6.2	4617177	6.8	104
GW501516	+	13394571	5.0	12500394	4.8	107
Propranolol	+	3172468	6.0	3189958	7.6	99
Toremifene	+	3873108	6.6	3851666	3.3	101

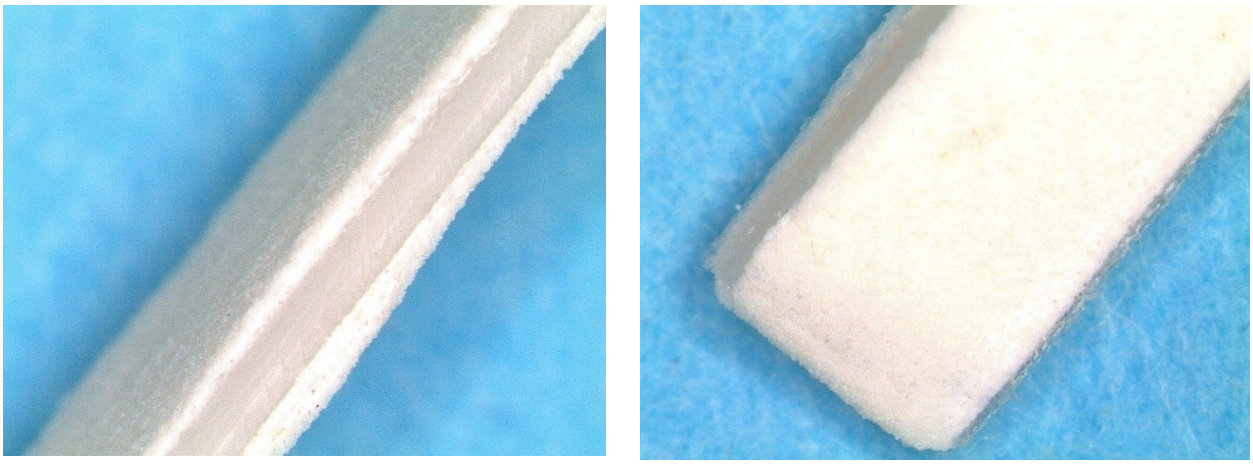


Figure S7. Microscope pictures of PAN-HLB thin films obtained by cutting pieces of 2.3 mm width from a coated flat PBT rectangular piece (8 x 10 cm).

Table S7. Transition ratios calculated from standards and from the tested matrices. Values were calculated by dividing the qualifier transition signal by the quantifier transition. Concentrations of up to 3 times LOQ values were considered for this calculation.

Compound	Transition ratios Qual/Quant				Deviation from standard, %		
	Standard	Urine	Plasma	Blood	Urine	Plasma	Blood
Morphine	0.71	0.74	0.80	0.69	5	14	2
Salbutamol	0.24	0.24	0.29	0.29	2	22	21
Nikethamide	0.26	0.26	0.22	0.26	1	13	1
Codeine	0.82	0.89	0.76	0.87	8	6	7
Benzoylecgonine	0.31	0.29	0.30	0.29	7	4	8
Amphetamine	0.24	0.26	0.20	0.25	7	19	3
Methamphetamine	0.26	0.20	0.24	0.24	25	10	10
Strychnine	0.68	0.64	0.58	0.71	6	15	5
Exemestane	0.72	0.68	0.82	0.75	4	14	5
Trenbolone	0.95	0.90	0.93	1.73	5	2	83
Metoprolol	0.66	0.74	0.71	0.78	13	9	19
Stanozolol	0.45	0.35	0.46	0.49	21	3	11
Clenbuterol	0.44	0.34	0.42	0.42	22	4	4
Bisoprolol	0.16	0.14	0.16	0.17	16	1	7
GW501516	0.26	0.25	0.26	0.26	4	1	2
Propanolol	0.92	0.92	0.88	0.93	0	5	0
Toremifene	0.10	0.10	0.09	0.09	1	12	9

Table S8. Average absolute recoveries obtained when using HLB thin films prepared on PBT support to extract from PBS spiked at 70 ng mL^{-1} (n=6 thin films).

Compound	No overcoated		Overcoated	
	Abs. recovery, %	RSD, %	Abs. recovery, %	RSD, %

Salbutamol	38.7	5.2	5.0	7.1
Codeine	83.2	4.4	8.4	5.1
Stanozolol	75.5	1.5	45.9	6.6
Clenbuterol	91.7	2.9	12.1	2.3
Bisoprolol	93.9	1.2	10.0	2.9
Propanolol	91.4	2.4	20.5	3.7