Supplementary Information for Chemical Communications

Solid phase microextraction (SPME)-transmission mode (TM) pushes down detection limits in direct analysis in real time (DART)*

*Germán Augusto Gómez-Ríos and Janusz Pawliszyn**

Department of Chemistry, University of Waterloo, Waterloo, ON, Canada

Email: janusz@uwaterloo.ca; Fax: 519-746-0435; Tel: 519-888-4567

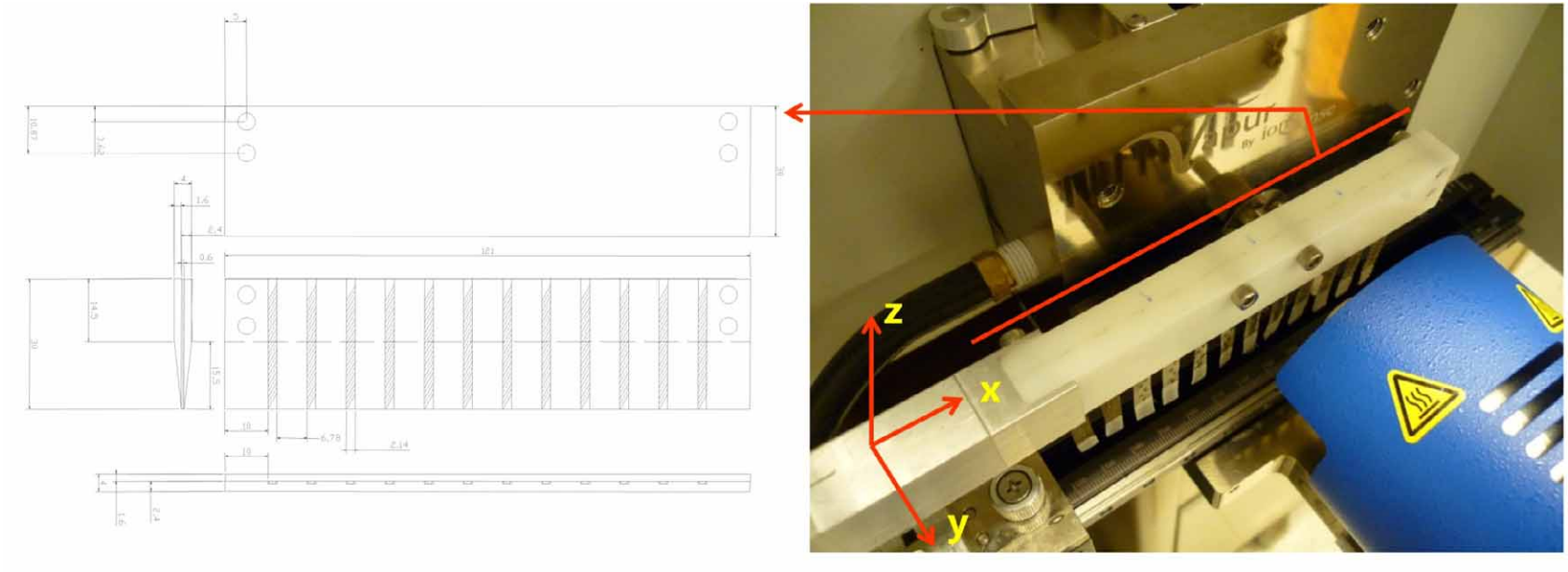


Figure S1 Schematic of UW-12 strips SPME-TMDART holder

The holder was developed at the machine shop of the University of Waterloo. It can be used to perform concomitant extractions on a 96 well-Concept autosampler (PAS technologies [1]), as well as automated and stable desorption/ionizations. The system is compatible with the automated rail commercialized by IonSense. Up to 12 SPME-TM devices can be easily installed/removed from the holder, and spatial position can be accurately adjusted on the Z and Y axis.

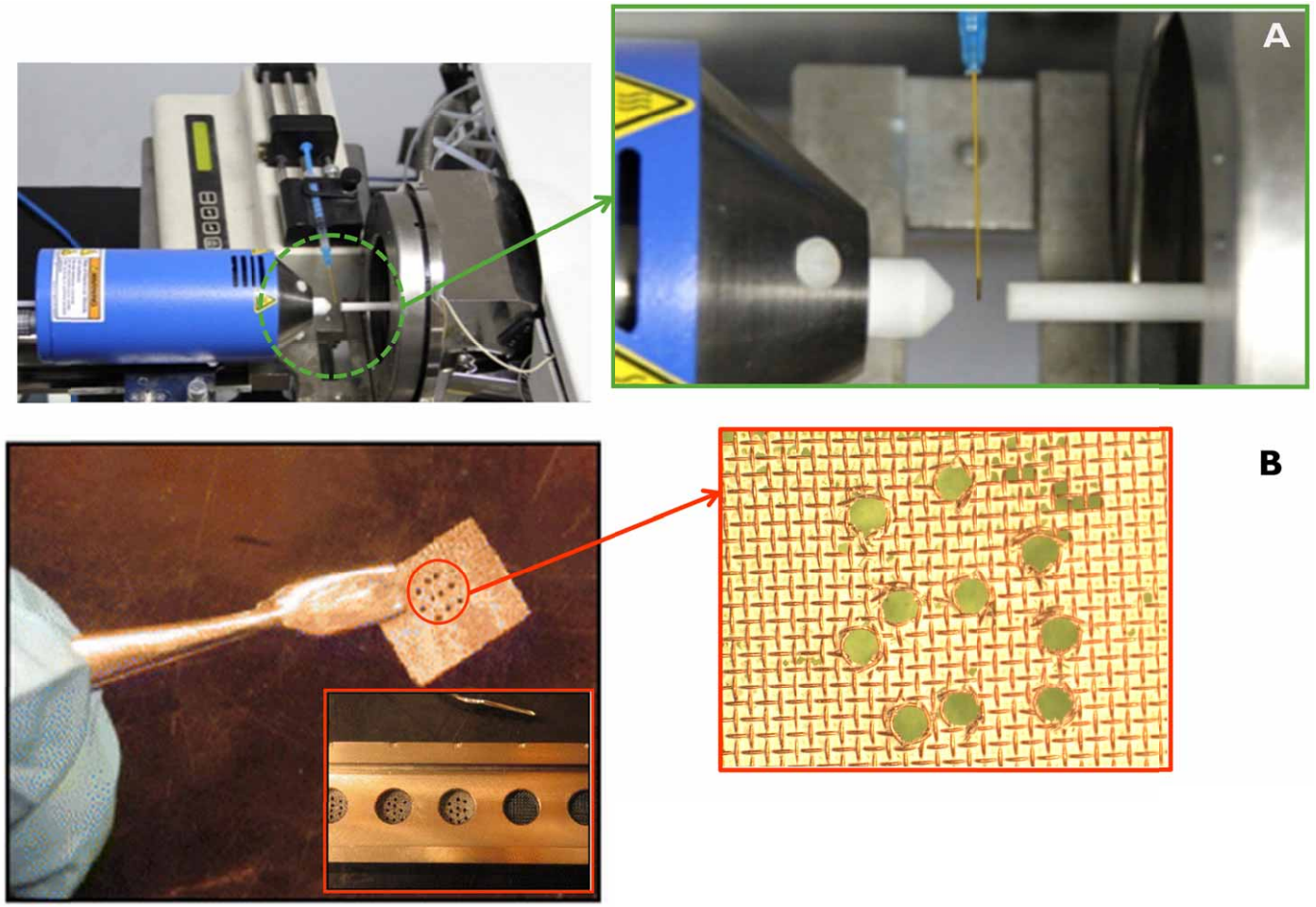


Figure S2A. In-Tube Solid-Phase Microextraction with Direct Analysis in Real Time Mass Spectrometry; image was adapted from the original source published by Wang and collaborators [2]. B. Thin-film solid-phase microextraction and direct analysis in real time; image was adapted from the original source published by Rodriguez-Lafuente and collaborators [3].

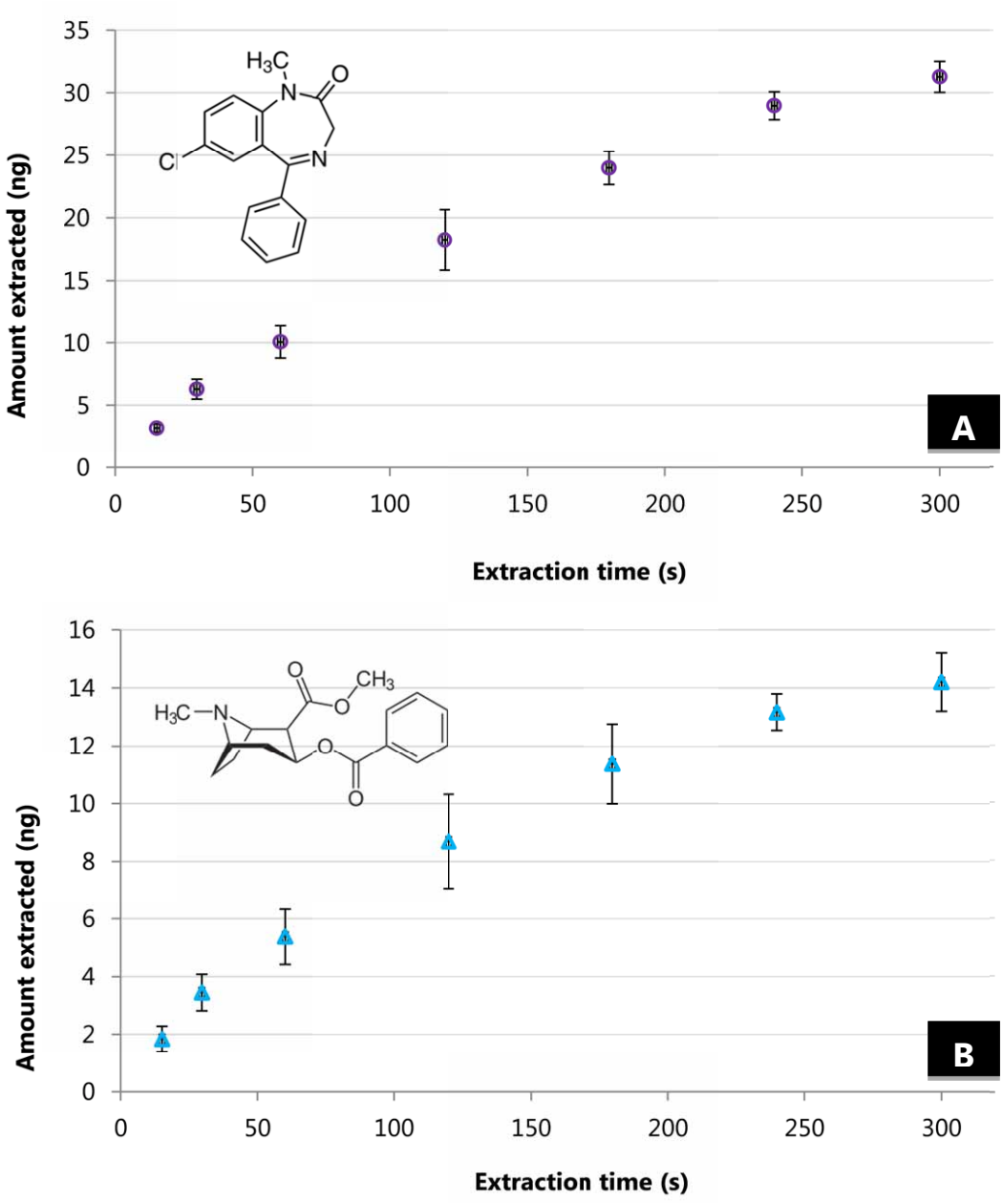


Figure S3 Extraction time profiles for A. diazepam and B. cocaine, respectively. Extractions were performed using a vortex agitator set-up at maximum speed (3200 rpm). Extractions from 1.5 mL of PBS spiked with 50 ppb of each analyte with 3 different blades (n = 6). Extracts were analyzed using Thermo TSQ LC-MS/MS on SRM mode.

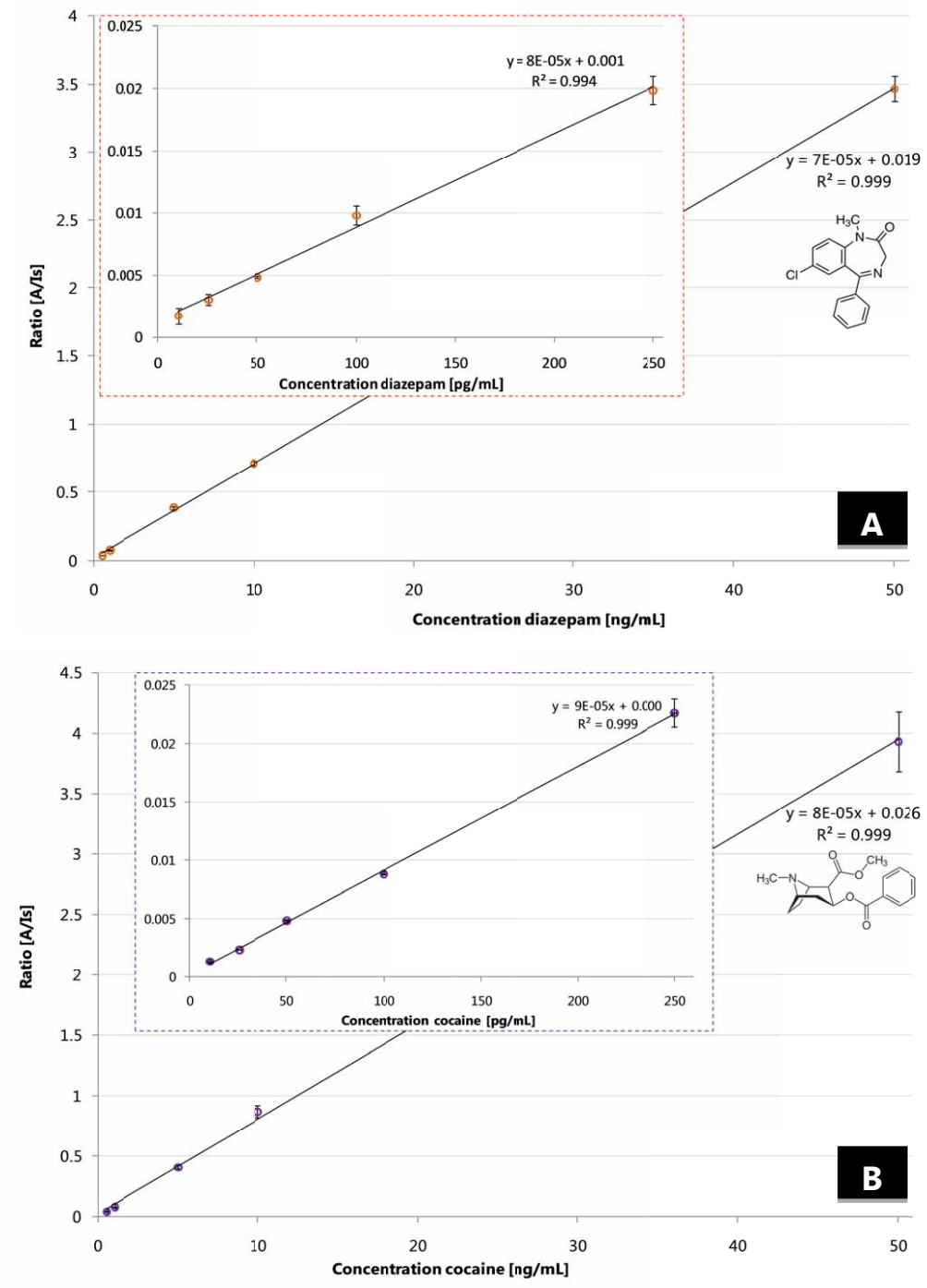


Figure S4 A. Quantitative analysis of PBS spiked with cocaine (10 pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_3] cocaine (12 ng mL^{-1}). **B.** Quantitative analysis of PBS spiked with diazepam (10 pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_5] diazepam (12 ng mL^{-1}).

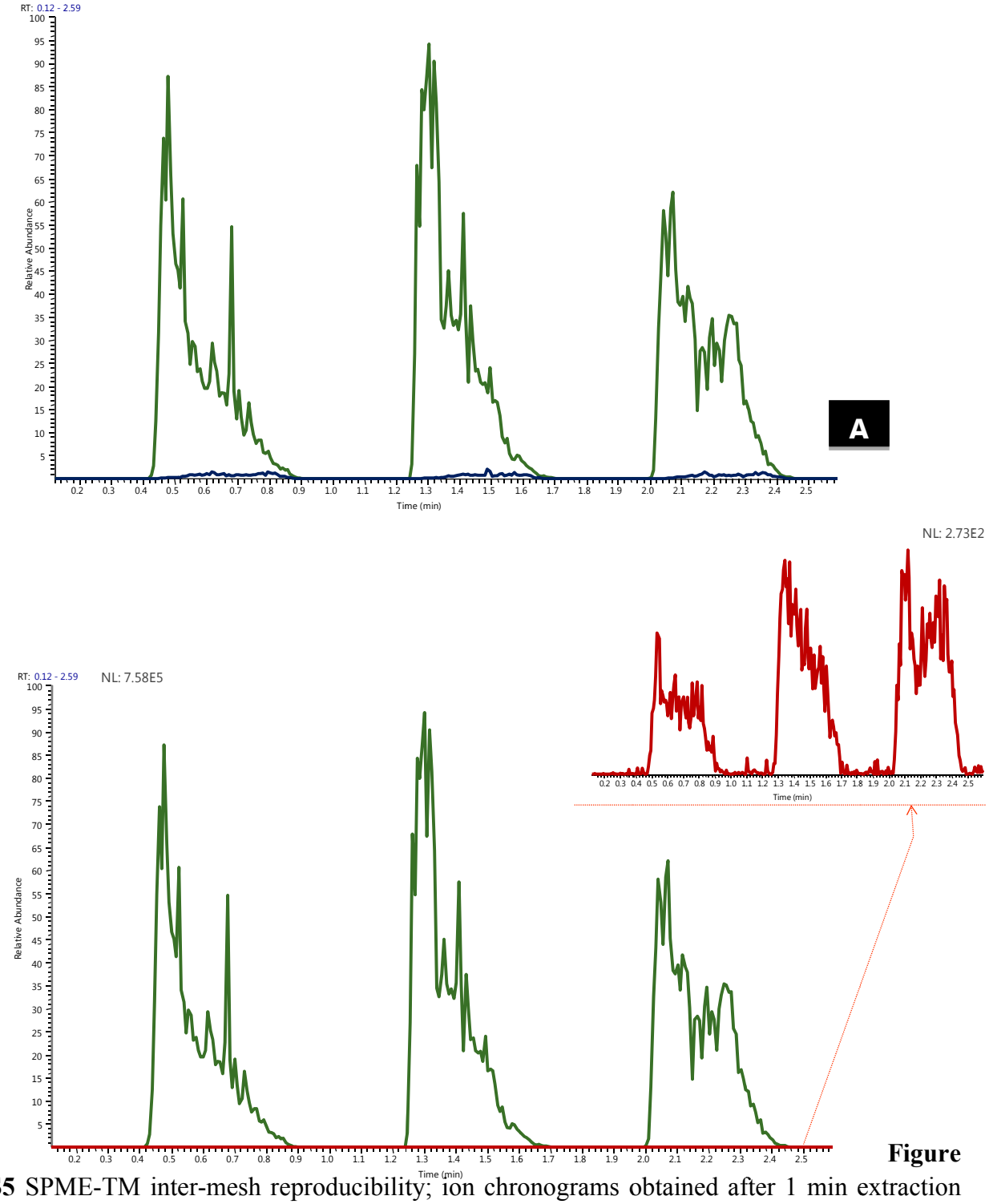


Figure S5 SPME-TM inter-mesh reproducibility; ion chronograms obtained after 1 min extraction from a solution spiked with 20 ppb of cocaine (green line) versus **A.** carry-over measured subsequently after the desorption/ionization cycle (blue line) and **B.** carry over measured after cleaning the SPME-TM device (red line) on 1.5 mL of a mixture of methanol, isopropanol and acetonitrile (50:25:25) for 30 minutes.

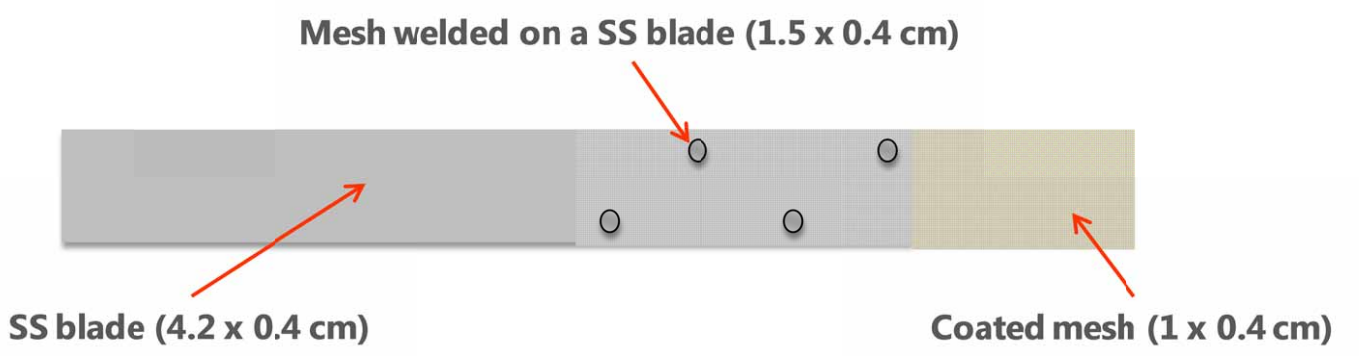


Figure S6 Scheme of the SPME-TM mesh-blade arrangement

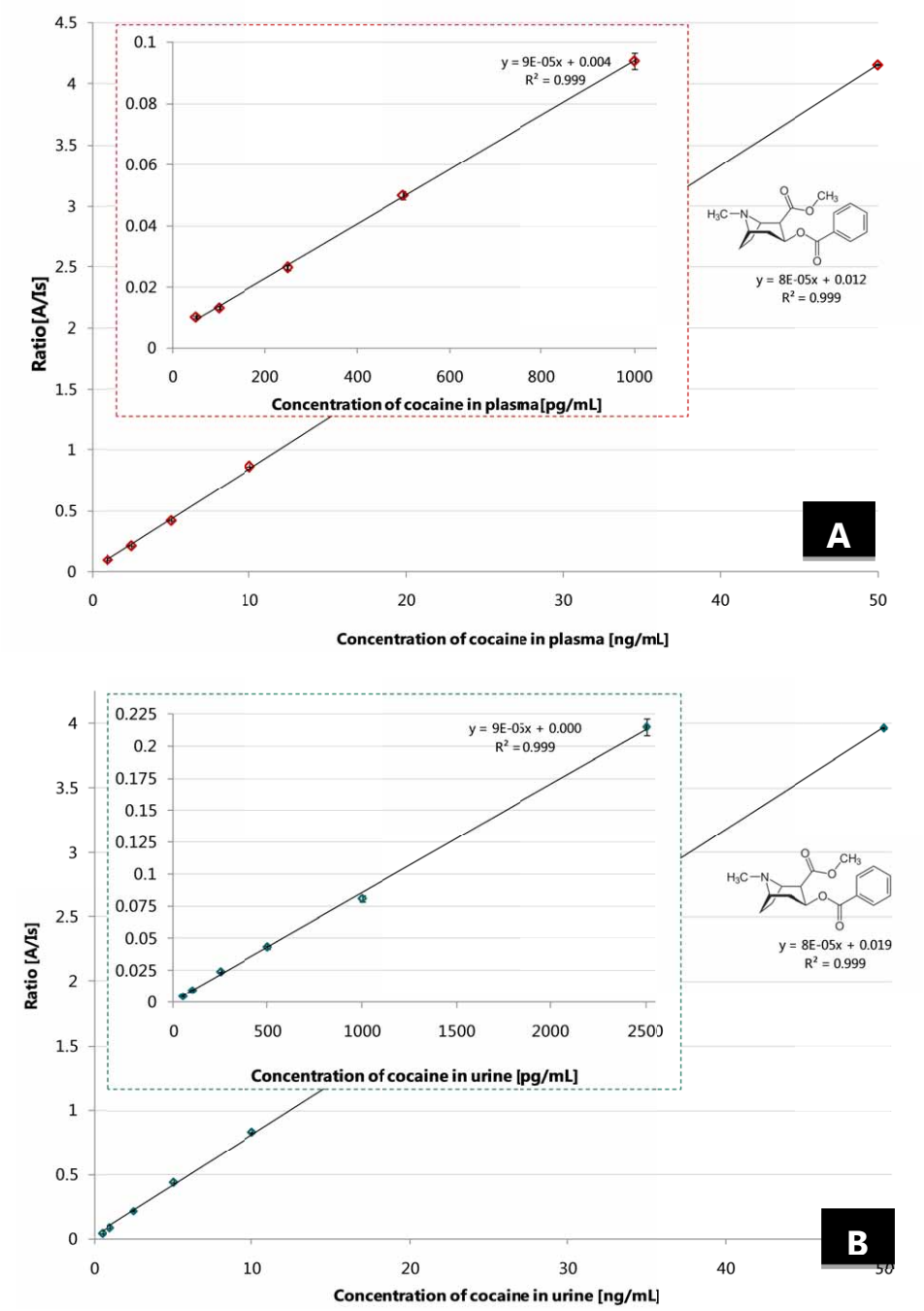
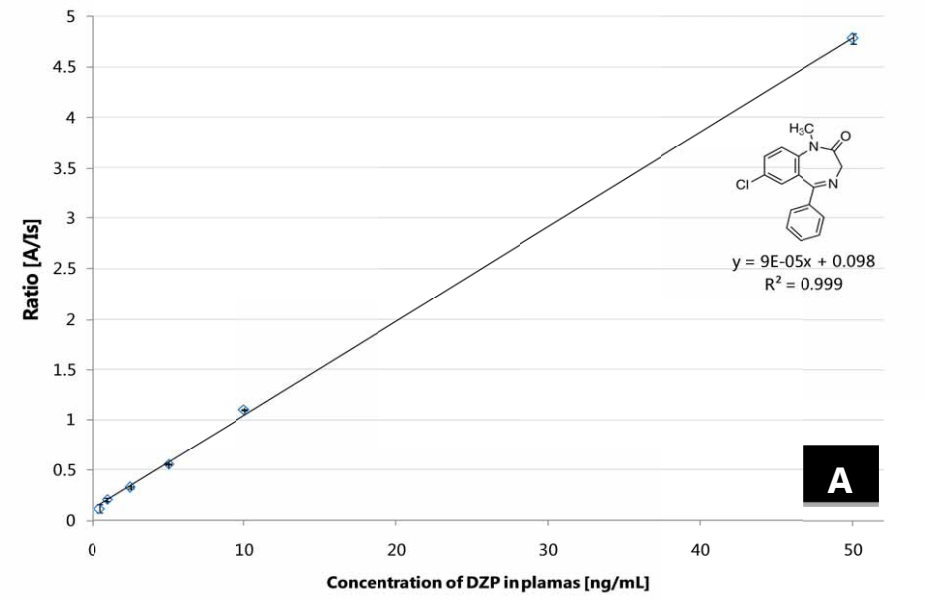


Figure S7-1 A. Quantitative analysis of plasma spiked with cocaine (50 pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_3] cocaine (12 ng mL^{-1}). **B.** Quantitative analysis of urine spiked with cocaine (50 pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_3] cocaine (12 ng mL^{-1}).



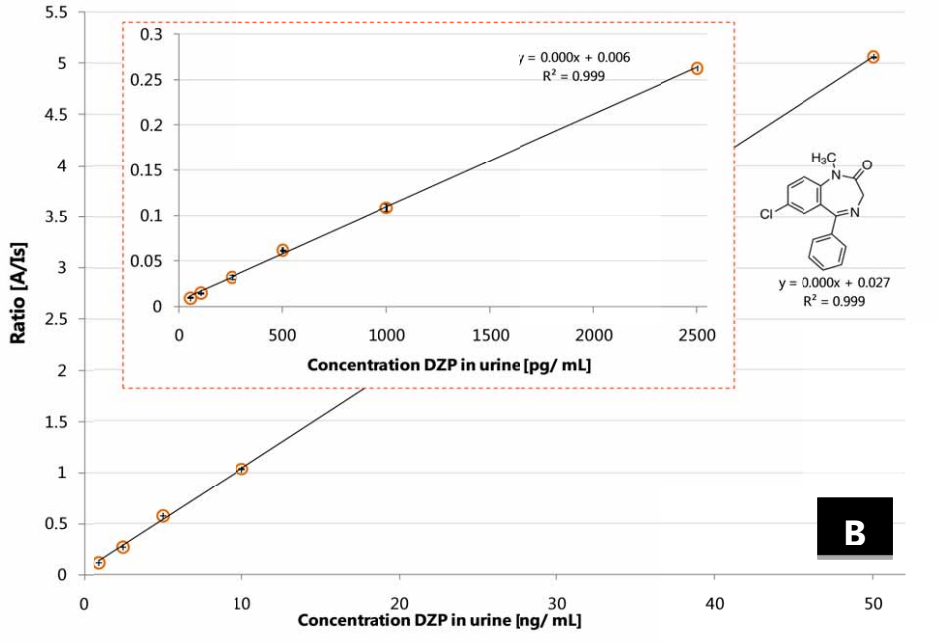
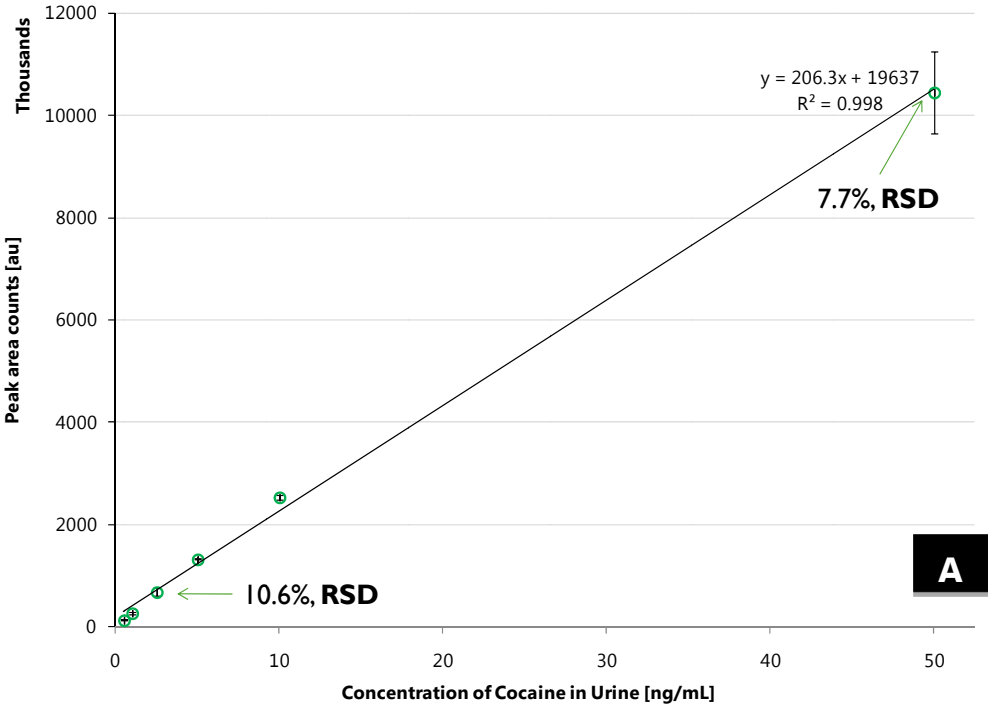


Figure S7-2 A. Quantitative analysis of plasma spiked with diazepam (500pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_5] diazepam (12 ng mL^{-1}). **B.** Quantitative analysis of urine spiked with diazepam (50 pg mL^{-1} to 50 ng mL^{-1}) and its isotopologue [D_5] diazepam (12 ng mL^{-1}).


Thousands
12000
10000
8000
6000
4000
2000
0
Peak area counts [au]
y = 206.3x + 19637
R² = 0.998
7.7%, RSD
10.6%, RSD
A
0
10
20
30
40
50
Concentration of Cocaine in Urine [ng/mL]

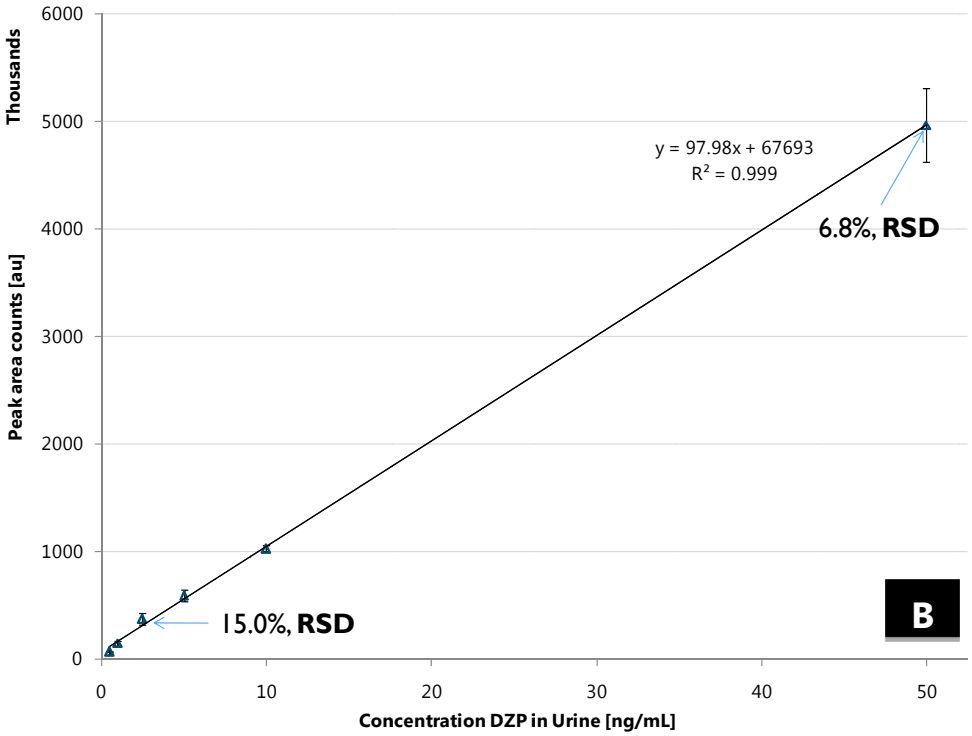

Thousands
6000
5000
4000
3000
2000
1000
0
Peak area counts [au]
y = 97.98x + 67693
R² = 0.999
6.8%, RSD
15.0%, RSD
B
0
10
20
30
40
50
Concentration DZP in Urine [ng/mL]

Figure S8 SPME-TM standard free calibration (n=3). **A.** Quantitative analysis of urine spiked with cocaine (500 pg mL^{-1} to 50 ng mL^{-1}) **B.** Quantitative analysis of urine spiked with diazepam (500 pg mL^{-1} to 50 ng mL^{-1}).

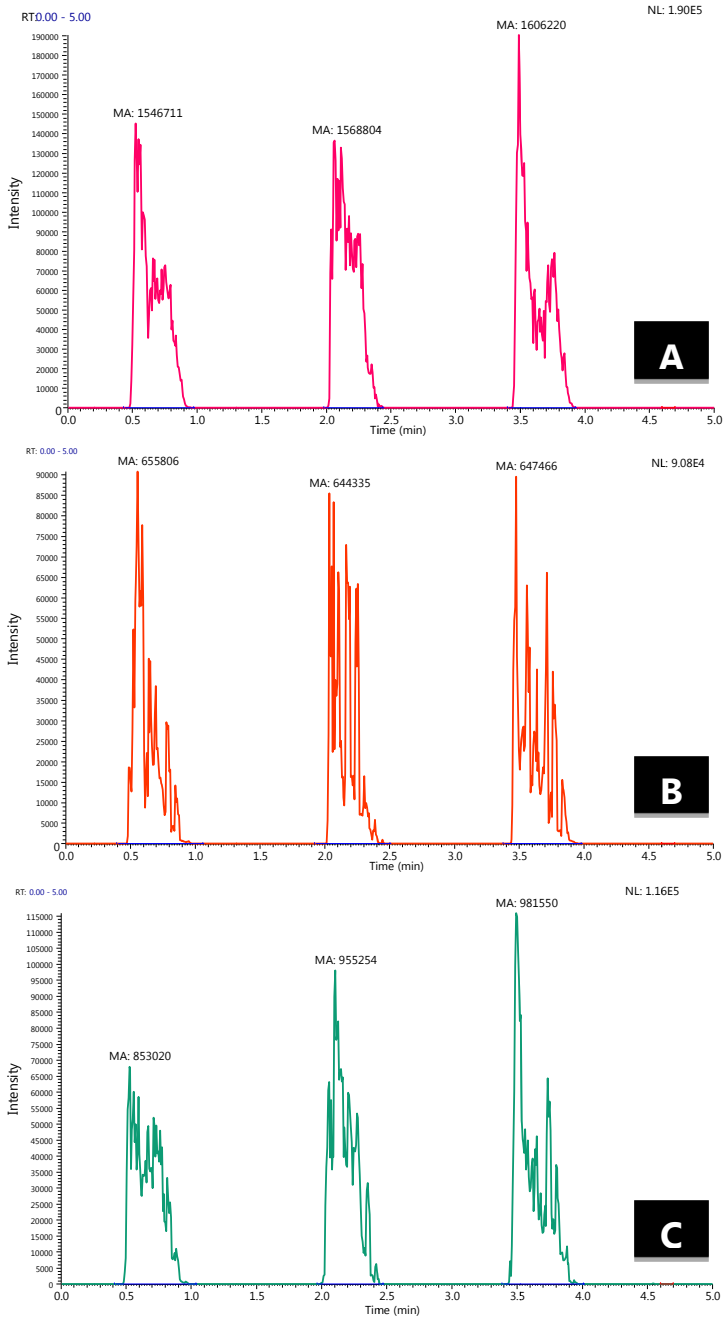


Figure S9 Ion chronograms of three controlled substances: heroin (**A**), propranolol (**B**), and stanozolol (**C**). 1 min extractions were performed using vortex agitator set-up at maximum speed (3200 rpm). Simultaneous extraction from 1.5 mL of PBS spiked with 20 ng mL^{-1} of 21 substances described on **Table S4**. Analyses were performed using a Thermo TSQ on MRM mode.

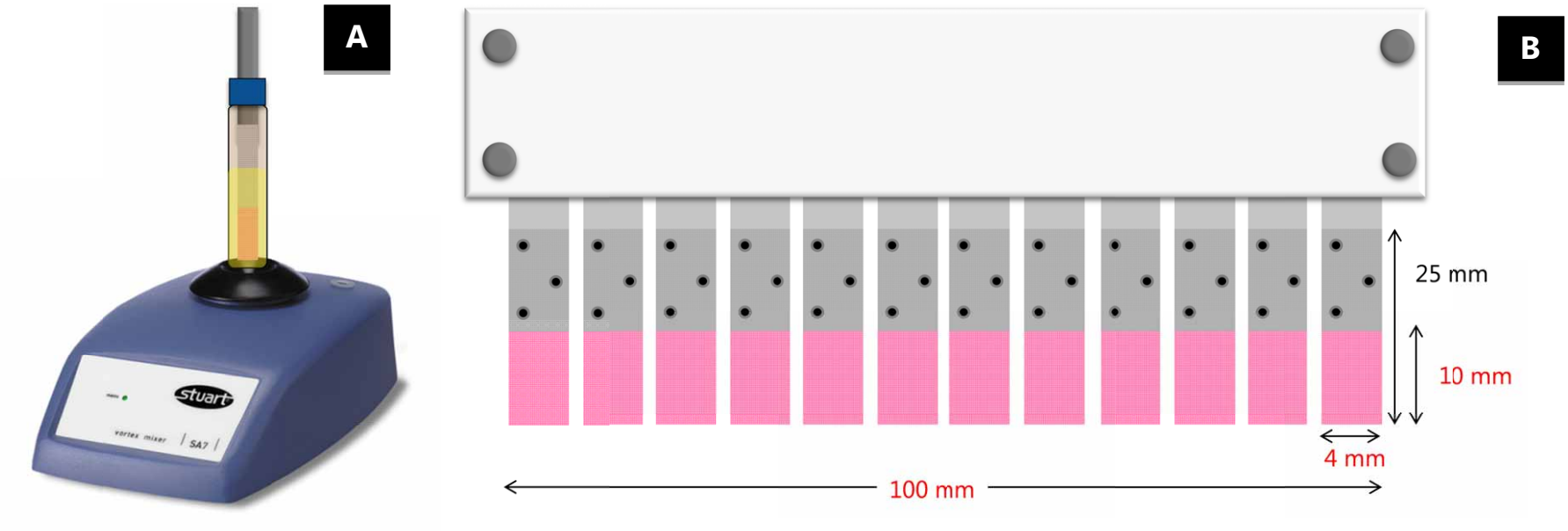


Figure S10 A. SPME-TM configuration for individual extractions and **B.** SPME-TM 12-strips configuration for high-throughput analysis using 96-Concept autosampler (PAS Technologies) [1].

Table S1-1 Inter- and intra-mesh reproducibility (n=36). Results are reported as ratio of analyte (diazepam) versus internal standard isotopologue [D_5] diazepam. 1 min extractions were performed using vortex agitator set-up at maximum speed (3200 rpm). Extraction from 1.5 mL of PBS spiked with 20 ng mL^{-1} of each substance. Analyses were performed using Thermo TSQ on SRM mode. SD, standard deviation. RSD, relative standard deviation.

Experiment	Mesh_1	Mesh_2	Mesh_3	Mesh_4	Mesh_5	Mesh_6	Mesh_7	Mesh_8	Mesh_9
Replicate 1	1.91	1.84	1.87	1.77	1.79	1.75	1.75	1.78	1.78
Replicate 2	1.87	1.80	1.77	1.80	1.80	1.80	1.75	1.77	1.71
Replicate 3	1.76	1.84	1.76	1.81	1.77	1.82	1.76	1.83	1.77
Replicate 4	1.80	1.84	1.94	1.86	1.83	1.82	1.77	1.74	1.78
Summary	1.83	1.83	1.84	1.81	1.80	1.80	1.76	1.78	1.76
SD	0.07	0.02	0.09	0.04	0.02	0.04	0.01	0.03	0.03
RSD	3.65	1.15	4.65	2.07	1.36	1.99	0.71	1.93	1.86

Table S1-2 Inter- and intra-mesh reproducibility (n=36). Results are reported as ratio of analyte (cocaine) versus internal standard isotopologue [D_3] cocaine 1 min extractions were performed using vortex agitator set-up at maximum speed (3200 rpm). Extraction from 1.5 mL of PBS spiked with 20 ng mL^{-1} of each substance. Analyses were performed using Thermo TSQ on SRM mode. SD, standard deviation. RSD, relative standard deviation.

Experiment	Mesh_1	Mesh_2	Mesh_3	Mesh_4	Mesh_5	Mesh_6	Mesh_7	Mesh_8	Mesh_9
Replicate 1	1.48	1.51	1.53	1.62	1.53	1.58	1.59	1.61	1.62
Replicate 2	1.54	1.54	1.59	1.57	1.62	1.58	1.59	1.64	1.59
Replicate 3	1.51	1.50	1.56	1.52	1.57	1.59	1.67	1.55	1.50
Replicate 4	1.55	1.52	1.47	1.54	1.45	1.53	1.61	1.55	1.53
Summary	1.52	1.52	1.54	1.57	1.54	1.57	1.62	1.59	1.56
SD	0.03	0.02	0.05	0.04	0.07	0.03	0.04	0.04	0.05
RSD	2.07	1.00	3.29	2.77	4.69	1.73	2.23	2.76	3.32

Table S2-1 Inter- and intra-mesh carry-over (n=36). Results are reported as ratio of analyte (diazepam) versus internal standard isotopologue [D_5] diazepam. 1 min extractions were performed using vortex agitator set-up at maximum speed (3200 rpm). Extraction from 1.5 mL of PBS spiked with 20 ng mL^{-1} of each substance. Analyses were performed using Thermo TSQ on SRM mode. SD, standard deviation. RSD, relative standard deviation.

Experiment	Average [A/Is]	SD	RSD [%]	% Carryover DART [A2/A1]	% Carryover solvent [A2/A1]
Replicate 1	1.81	0.06	3.15	7.25	0.46
Replicate 2	1.79	0.04	2.40	4.38	0.33
Replicate 3	1.79	0.03	1.93	4.34	0.27
Replicate 4	1.82	0.06	3.10	3.91	0.23
Summary	1.80	0.05	2.71	4.97	0.32

Table S2-2 Inter- and intra-mesh carry-over (n=36). Results are reported as ratio of analyte (cocaine) versus internal standard isotopologue [D_3] cocaine. 1 min extractions were performed using vortex agitator set-up at maximum speed (3200 rpm). Extraction from 1.5 mL of PBS spiked with 20 ng mL^{-1} of each substance. Analyses were performed using Thermo TSQ on SRM mode. SD, standard deviation. RSD, relative standard deviation.

Experiment	Average [A/Is]	SD	RSD [%]	% Carryover DART [A2/A1]	% Carryover solvent [A2/A1]
Replicate 1	1.56	0.05	3.35	3.06	0.50
Replicate 2	1.58	0.03	2.20	1.80	0.11
Replicate 3	1.55	0.05	3.45	2.04	0.06
Replicate 4	1.53	0.05	3.02	2.53	0.06
Summary	1.56	0.05	3.19	2.36	0.18

Table S3 Typical limits of quantification (LOQ) obtained for cocaine using LC-MS/MS, GC-MS and DART-MS/MS with diverse approaches versus the results presented in this article.

Sample	LOQ [ng/mL]	Sample Preparation	Instrument	Authors	Reference
Urine	3.5	SPE	LC-MS/MS	Berg et al.	4
Urine	5	TFME	LC-MS/MS	Boyaci et al.	5
Urine	0.1	SPME-TM	DART-MS/MS	Rodríguez-Lafuente et al.	3
Urine	0.002	SPME-TM	DART-MS/MS	*	-
Serum	0.5	on-line SPE	LC-MS/MS	Bouzas et al.	6
Plasma	25	SPME	GC-MS	Alvarez et al.	7
Plasma	0.005	SPME-TM	DART-MS/MS	*	-

Table S4 MS/MS parameters used for the analysis of 21 WADA controlled substances in positive mode, as well as instrumental response of C_{18}-PAN SPME-TM tandem mass spectrometry analysis. Results are based on the integrated peak area obtained for a 20 ng mL^{-1} solution in PBS. Average peak area (n=3). LOD*, limit of detection estimated.

#	Compound name	Log P	Parent ion (m/z)	Product ion (m/z)	S-lenses	Collision energy	Polarity	LOD* [pg/mL]
1	Amphetamine	1.76	136.099	91.114	17	36	+	112
2	Methamphetamine	2.07	150.112	91.120	19	45	+	20
3	Nikethamide	0.33	179.100	108.102	18	76	+	17
4	Salbutamol	0.64	240.143	148.103	18	59	+	1474
5	Propranolol	3.48	260.123	116.138	17	89	+	31
6	Metoprolol	1.60	268.140	116.146	18	94	+	108
7	Trenbolone	2.27	271.133	165.106	56	97	+	31
8	Clenbuterol	2.61	277.068	203.049	15	70	+	13
9	Testosterone	3.32	289.157	97.123	21	91	+	10
10	Exemestane	3.70	297.173	121.118	19	72	+	17
11	Codeine	1.20	300.105	152.092	64	104	+	46
12	Cocaine	2.30	304.142	182.173	18	87	+	2
13	Bisoprolol	2.14	326.160	116.135	17	102	+	45
14	6-acetylmorphine	0.42	328.126	165.092	37	122	+	21
15	Stanozolol	5.53	329.229	81.108	44	130	+	22
16	Strychnine	1.93	335.155	184.129	36	136	+	33
17	6-acetylcodeine	2.08	342.124	165.092	45	165	+	7
18	Formoterol	2.20	345.133	121.090	32	85	+	831
19	Heroin	1.52	370.133	165.097	48	119	+	13
20	Toremifene	6.80	406.210	72.167	24	108	+	42
21	GW501516	6.29	454.091	257.068	29	108	+	352

References

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