Supporting Information

Biocompatible-Solid Phase Microextraction (Bio-SPME)-nano-electrospray ionization (nano-ESI): an unexploited tool in bioanalysis

Germán Augusto Gómez-Ríos, Nathaly Reyes-Garcés, Barbara Bojko, Janusz Pawliszyn*

Department of Chemistry, University of Waterloo, Ontario, Canada N2L 3G1

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

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

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 selective reaction monitoring transitions used to quantify each model compound; inter-emitter device reproducibility results; figures of merit for analysis of PBS, blood, and urine; pictures of the ion source and of vials used for sampling of small sample volumes.

Section 1

Brief summary of direct to MS applications up to date using SPME fibres

The direct interface of SPME fibers with MS analyzers has been investigated since the late 1990s[1–4]. For this purpose, different strategies have been followed by several groups around the world. Some of the most relevant approaches involve placing the fiber at the electron impact region of the MS[5]; thermally desorbing an SPME/Electrochemistry (SPME/EC) fibre on a GC injection port prior to its direct coupling to an Ion-Trap MS via a deactivated fused silica column[3]; thermally desorbing fibers prior to Inductively Coupled Plasma-MS (ICP-MS) analysis[4,6]; desorbing fibers on a solvent with high affinity for the analytes of interest preceding atmospheric pressure ionization (API), either by ESI[7]or APCI[8]; ablating analytes from the fiber surface with a laser either at vacuum[9] or at atmospheric pressure[10–12]; and interfacing the fibers with AMS instrumentation, for instance DESI[13,14] or DART[15,16]. An interest summary of these couplings can be found on review published by Deng and collaborators[17].

Section 2

Biological samples

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.

Section 3Preliminary experiments for small volume sampling

In order to challenge the new Bio-SPME-nano-ESI platform, it was used for the quantitative analysis in volumes ranging between 10 and 1500 μL. As shown in Figure S6, to ensure that the entire fiber remained immersed in the sample, glass vials with a fused-in conical insert and fibers with a coating length of 4 mm were used. At the outset, we wanted to demonstrate that independently of sample volume, the ratio of analyte to internal standard extracted by the fiber remained constant and that good signal was attained. As can be seen in Figure S7, 1-minute extractions from PBS spiked with cocaine and diazepam at 25 ng mL-1 yielded non-statistical differences among the five volumes evaluated (i.e. 10, 50, 100, 300, and 1500 μL). Therefore, based on these results, we proceeded to perform similar experiment in human whole blood (please see Figure 5 in main document).

Table S1 Target analytes, manufacturers, and SRM transitions monitored for each model compound in positive ionization mode.

Compound	Manufacturer	Matrix	Log P	Protein binding [%]	Parent [m/z]	Fragment [m/z]	Collision Energy	S-Lens
Diazepam	Cerilliant [1]	PBS	2.91	98	285.050	193.113	32	102
Diazepam-d_5	Cerilliant	PBS	-	-	290.075	198.179	33	113
Cocaine	Cerilliant	PBS	3.08	5	304.122	182.139	19	90
Cocaine-d_3	Cerilliant	PBS	-	-	307.140	185.190	20	91
Methadone	Cerilliant	Urine	4.20	90	310.189	265.281	14	82
Methadone-d_3	Cerilliant	Urine	-	-	313.199	268.304	15	77
Codeine	Cerilliant	Urine	1.20	-	300.136	152.146	63	124
Codeine-d_3	Cerilliant	Urine	-	-	303.139	152.135	64	118
Salbutamol	Cerilliant	Urine	0.01	-	240.146	148.179	17	70
Salbutamol-d_3	Cerilliant	Urine	-	-	243.144	151.170	19	67
Oxycodone	Cerilliant	Urine	1.67	45	316.121	241.215	29	100
Oxycodone-d_3	Cerilliant	Urine	-	-	319.140	244.246	28	100
Amitriptyline	Sigma-Aldrich [2]	Blood	4.92	≥ 90	278.148	233.461	16	86
Amitriptyline-d_6	TRC [3]	Blood	-		284.140	233.473	19	82
Imatinib	Sigma-Aldrich	Blood	2.48	95	494.180	394.790	26	123
Imatinib-d_3	TRC	Blood	-	-	497.204	394.785	28	128

1. Cerilliant (Round Rock, TX, USA), 2. Sigma-Aldrich (Sigma-Aldrich (Oakville, ON, Canada), 3.TRC, Toronto Research Chemicals (Toronto, ON, Canada); Log P, logarithm of its partition coefficient between n-octanol and water. Scan time was 100 ms for all analytes, with a total spraying time of 45 seconds per replicate at 1.3 kV and 3 mm distance from the MS ion-transfer capillary. All the experiments were performed using a Thermo TSQ Vantage (Thermo Scientific, San Jose, USA).

Table S2 Inter-emitter reproducibility of commercial emitter (commercialized by New Objective) suitable for Bio-SPME-nano-ESI experiments. RSD, Relative Standard Deviation (n=3).

Compound	RSD [%] n=3		
	Econo10	BG75-2	BG75-4
Cocaine	6.1	5.8	6.1
Cocaine-d3	6.0	5.5	6.0
Ratio	0.2	0.4	0.2
Diazepam	3.3	12.7	3.3
Diazepam-d5	2.0	11.3	2.0
Ratio	2.0	1.5	2.0

Table S3 Experimental replicates using a single nano-ESI emitter. (n=4). Signals correspond to 1 min extraction from 1.5 mL of PBS spiked with 75 ng mL^{-1} of analyte. Extractions were performed using a 15 mm Bio-SPME mix mode fiber. Desorption volume was 4 µL and desorption time was 5 minutes. Spraying voltage was 1.3 kV with an acquisition time of 0.9 min.

Compound	Replicate [area counts, au]			Average	SD	RSD [%]
	2	3	4			
Diazepam	25026413	27691475	29623102	27446997	2308076	8.4
Diazepam-d5	4120991	4486829	4756566	4454795	318996	7.2
Ratio	6.1	6.2	6.2	6.2	0.08	1.3
Cocaine	177866820	175990773	173211262	175689618	2342344	1.3
Cocaine-d3	31055950	29983291	29548704	30195982	775806	2.6
Ratio	5.7	5.9	5.9	5.8	0.08	1.4

Table S4 Figures of merit, concomitant analysis of diazepam and cocaine in PBS

Compound	Concentration accuracy (%)			LOD [pg/mL]	LOQ [pg/mL]
	300 [pg/mL]	7.5 [ng/mL]	200 [ng/mL]		
Diazepam	91 ± 3.9	97 ± 0.1	98 ± 0.9	34	102
Cocaine	92 ± 2.3	97 ± 1.3	87 ± 2.9	11	34

Table S5 Figures of merit, concomitant analysis of salbutamol, codeine, methadone, and oxycodone in pooled urine.

Compound	Accuracy concentration Level (%)		LOD [ng/mL]	LOQ [ng/mL]
	2.5 [ng/mL]	75 [ng/mL]		
Salbutamol	-	90 ± 1.4	1.1	3.3
Codeine	-	89 ± 1.2	2.1	6.4
Methadone	90 ± 1.8	89 ± 1.4	0.1	0.2
Oxycodone	-	90 ± 0.4	1.4	4.1

Table S6 Figures of merit, concomitant analysis of amitriptyline and imatinib in whole human blood.

Compound	Accuracy (%)	LOD [ng/mL]	LOQ [ng/mL]
	100 [ng/mL]		
Amitriptyline	110 ± 1.8	1.6	4.9
Imatinib	107 ± 1.0	2.3	7.0

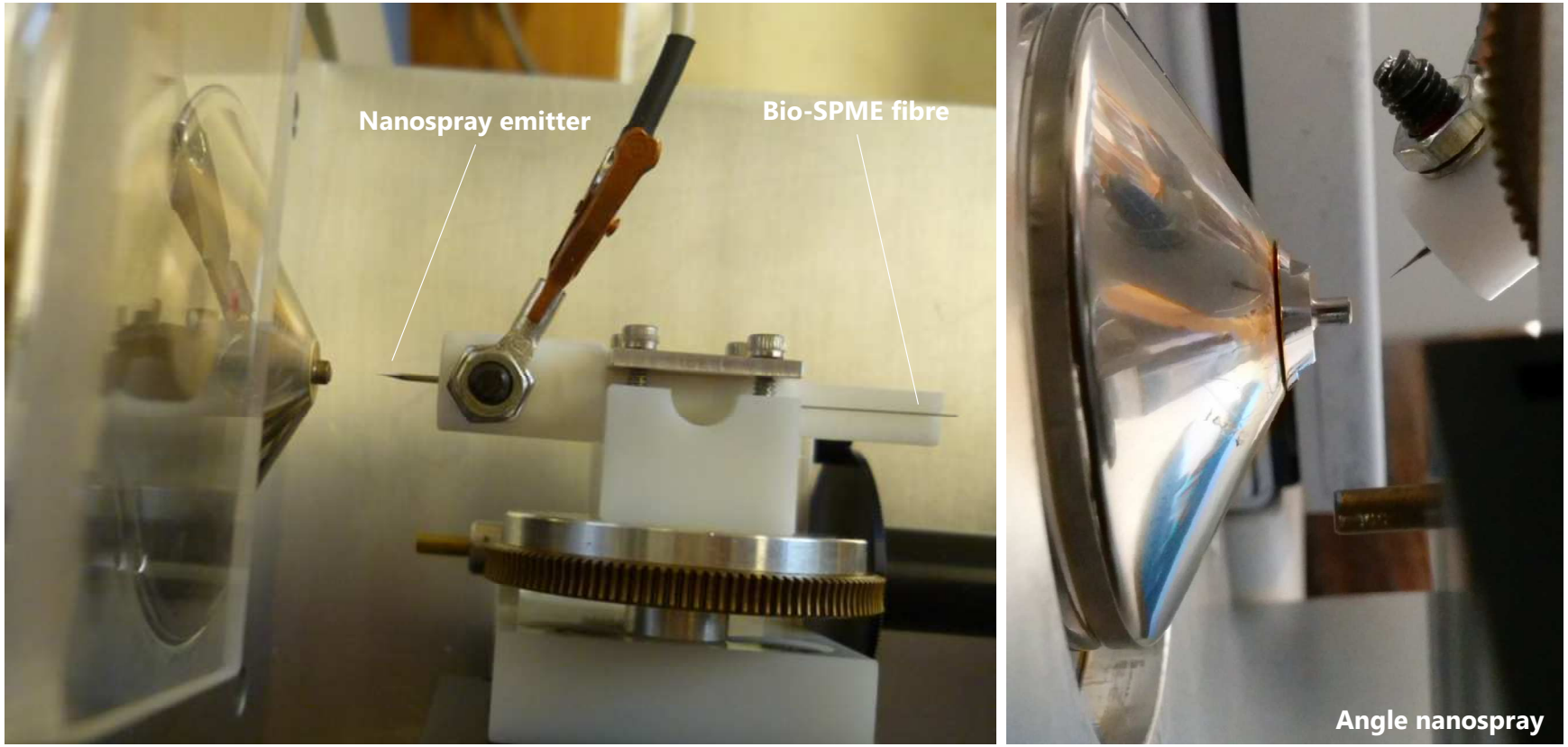

Figure S1 In-house ionization source for Bio-SPME-nano-spray. The 3D-moving stage (Newport Corporation, Irvine, CA) not only adjusts the position with a precision of 0.02 mm in each dimension (25 mm moving path), but also tunes the spraying tip at different angles on the Z dimension (± 0.01° per moving mark). In order to ensure optimum ion transmission, the nano-spray emitter was positioned at 3 mm from the ion-transfer capillary.

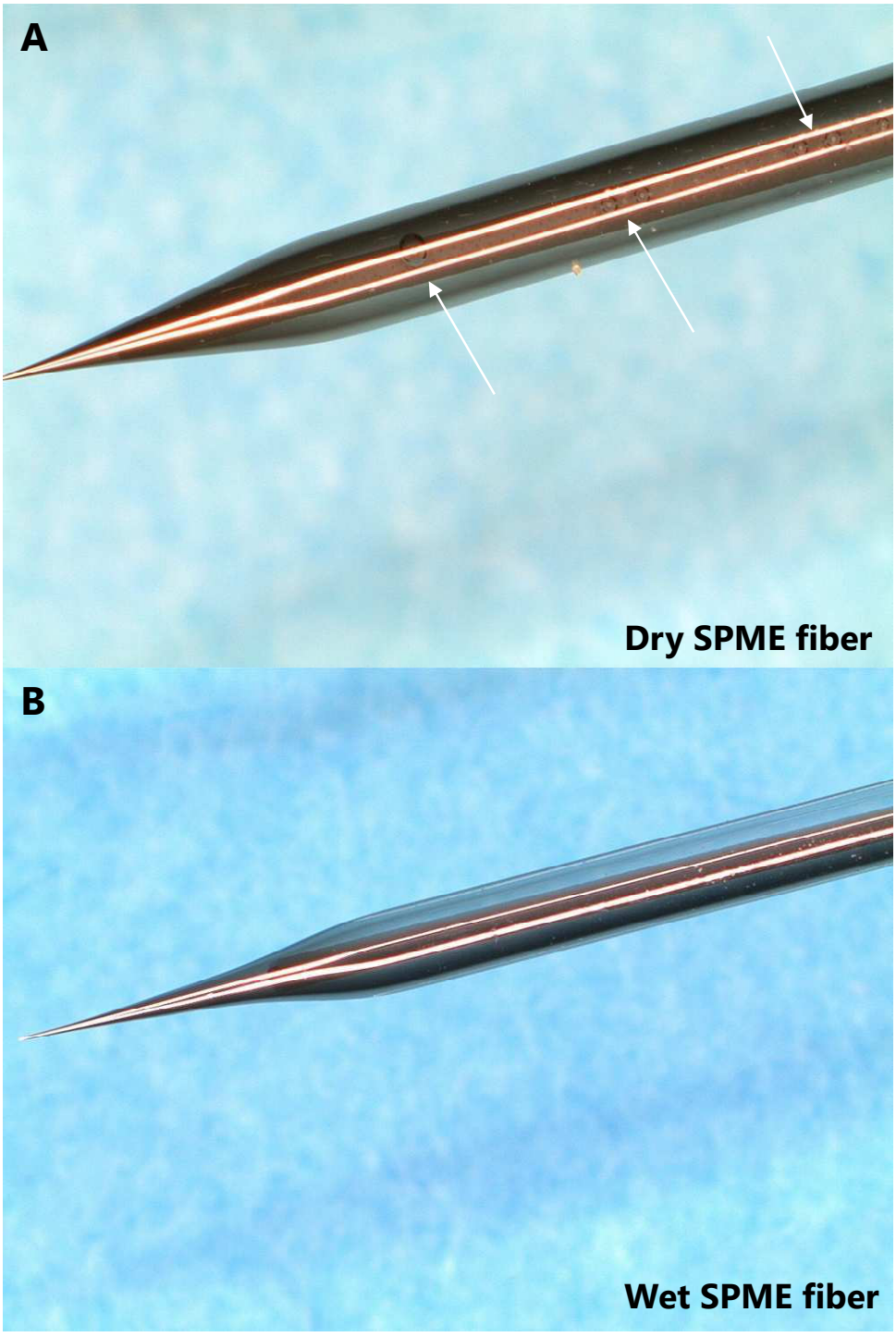

Figure S2 15 mm dry SPME fiber (**A**) versus wet SPME fiber (**B**) inserted into a nano-ESI emitter filled with 4 µL of methanol. Bubbles are indicated by white arrows.

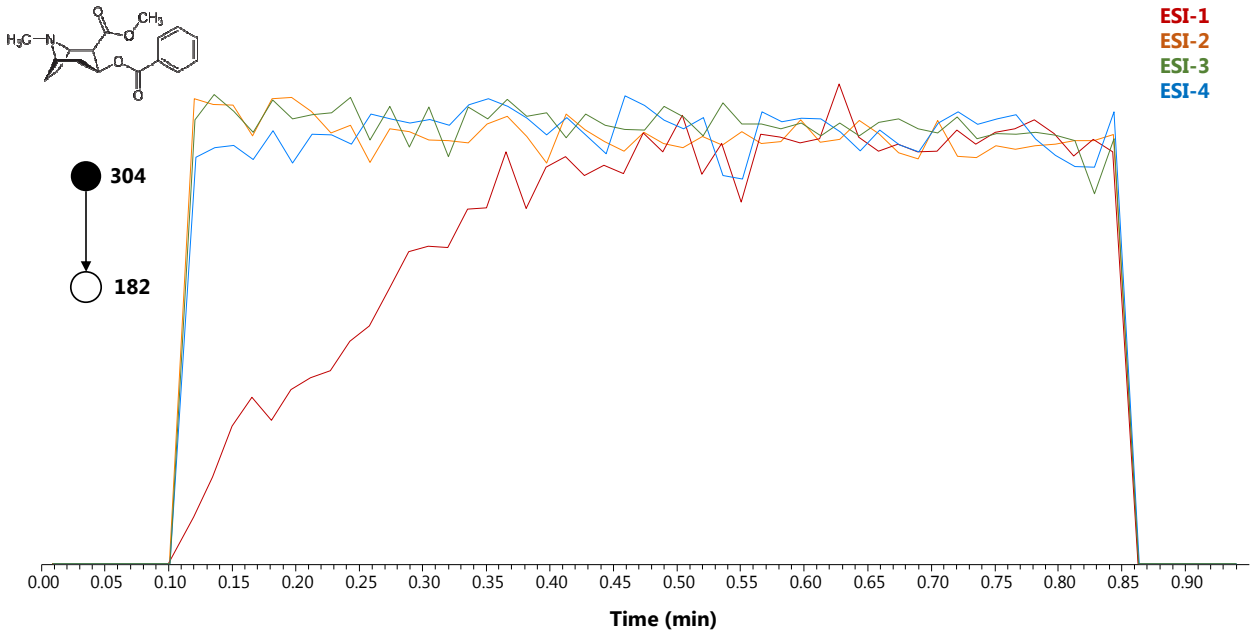

Figure S3 Cocaine ion-chronograms obtained using the same nano-ESI emitter (n=4). Signals correspond to 1 min extraction from 1.5 mL of PBS spiked with 75 ng mL^{-1} of analyte. Extractions were performed using a 15 mm BioSPME mix mode fiber. Desorption volume was 4 µL and desorption time was 5 minutes. Spraying voltage was 1.3 kV with an acquisition time of 0.9 min.

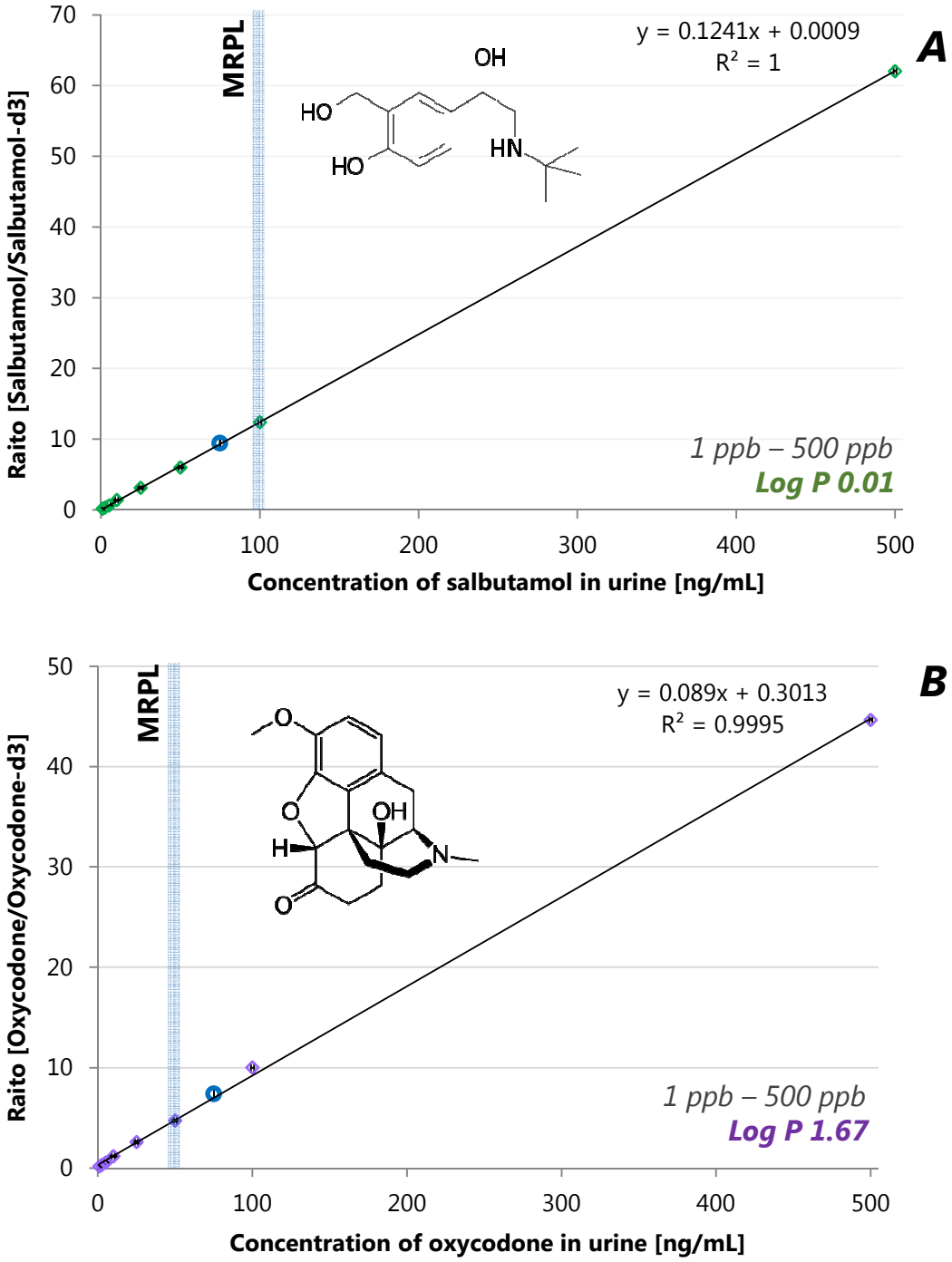

Figure S4 Quantitative analysis of urine spiked with salbutamol (1 ng ml^{-1} to 500 ng mL^{-1}) and its isotopologue [D_3] salbutamol (10 ng mL^{-1}). **B.** Quantitative analysis of urine spiked with oxycodone (1 ng ml^{-1} to 500 ng mL^{-1}) and its isotopologue [D_3] oxycodone (12 ng mL^{-1}). Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters. Blue circles represent the accuracy levels evaluated for both compounds. MRPL, Minimum Required Performance Level.

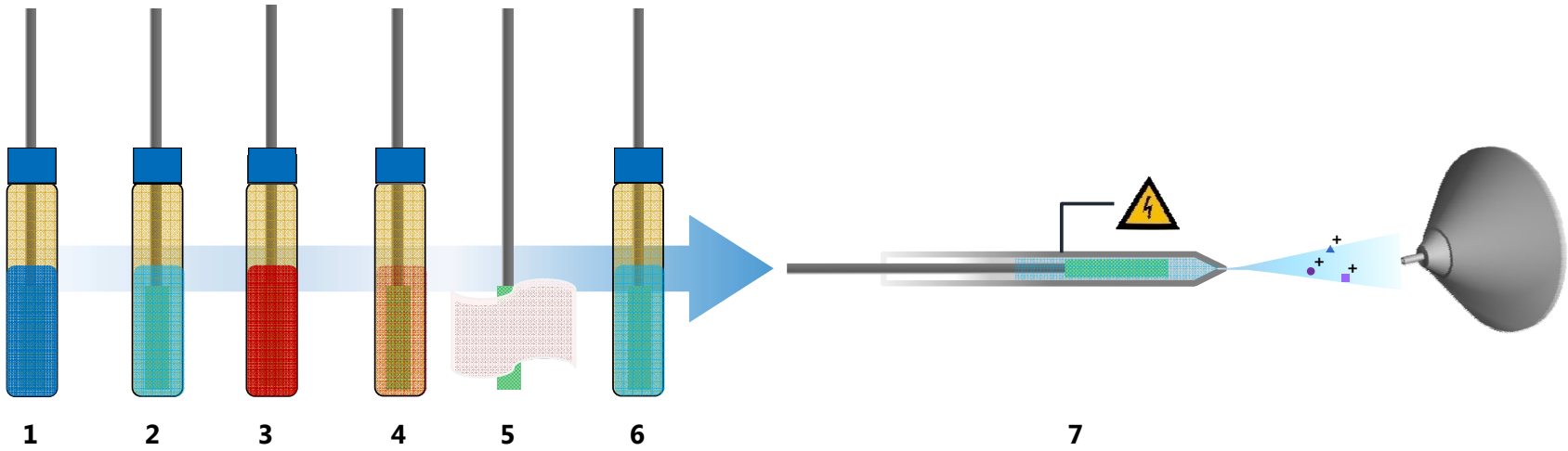

Figure S5 Experimental set up for Bio-SPME extraction from whole blood and desorption–ionization using nano-ESI-MS/MS. The analytical process can be summarized in 7 steps. **1.** Fiber pre-conditioning; **2.** Fiber rinsing in water to remove excess of methanol (10s); **3.** Extraction from whole blood (2 min); **4.** Fiber rinsing in water to remove cells and proteins attached to coating surface (5s); **5.** Fiber cleaning with a piece of Kim wipe tissue (5s); **6.** Additional rising step to remove small particles that might have remained attached to the surface (5s); **7.** Desorption/ionization step using acidified methanol (0.1% FA).

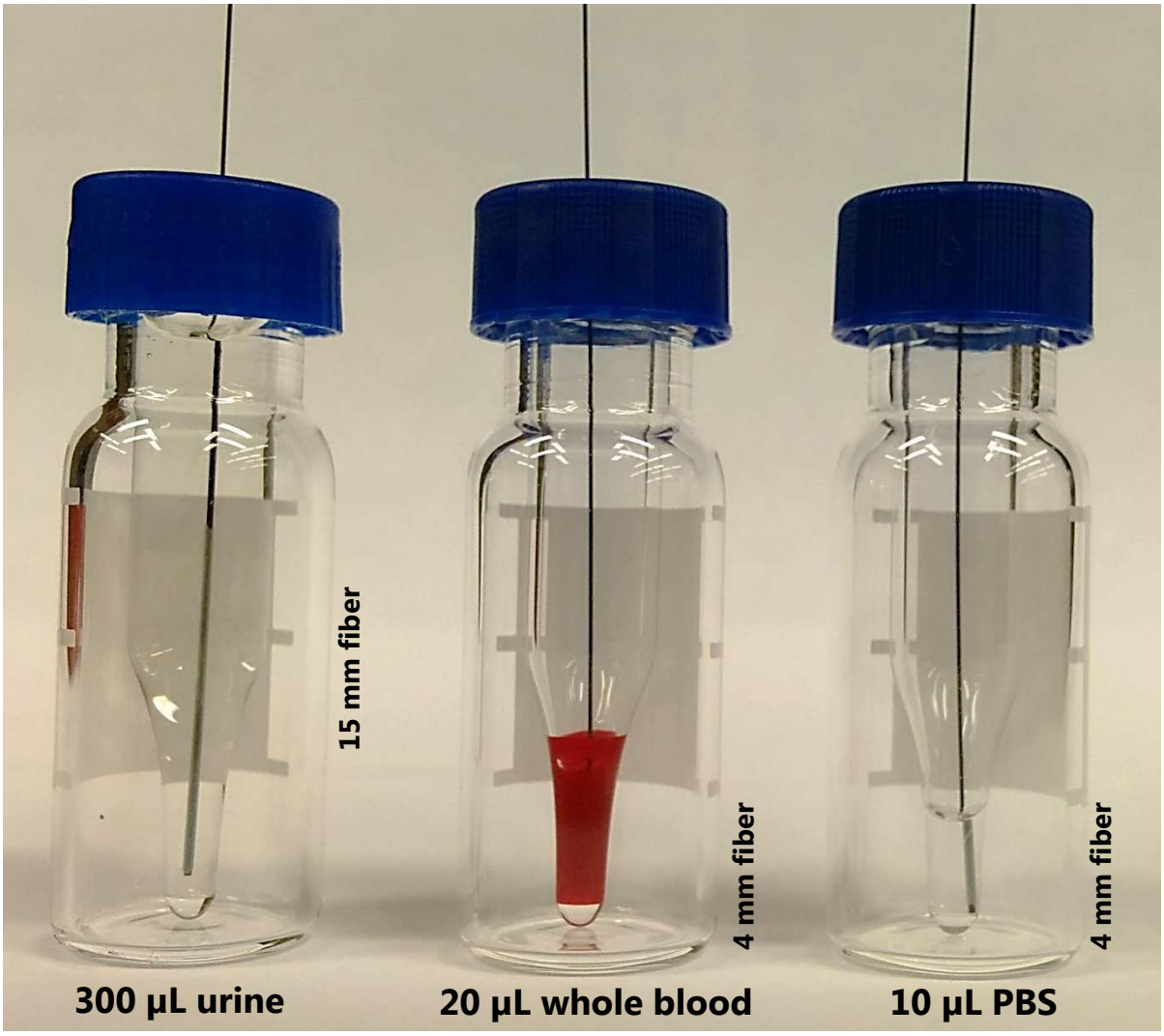

Figure S6 Small sample volume analysis using 15 and 4 mm mix-mode Bio-SPME fibers.

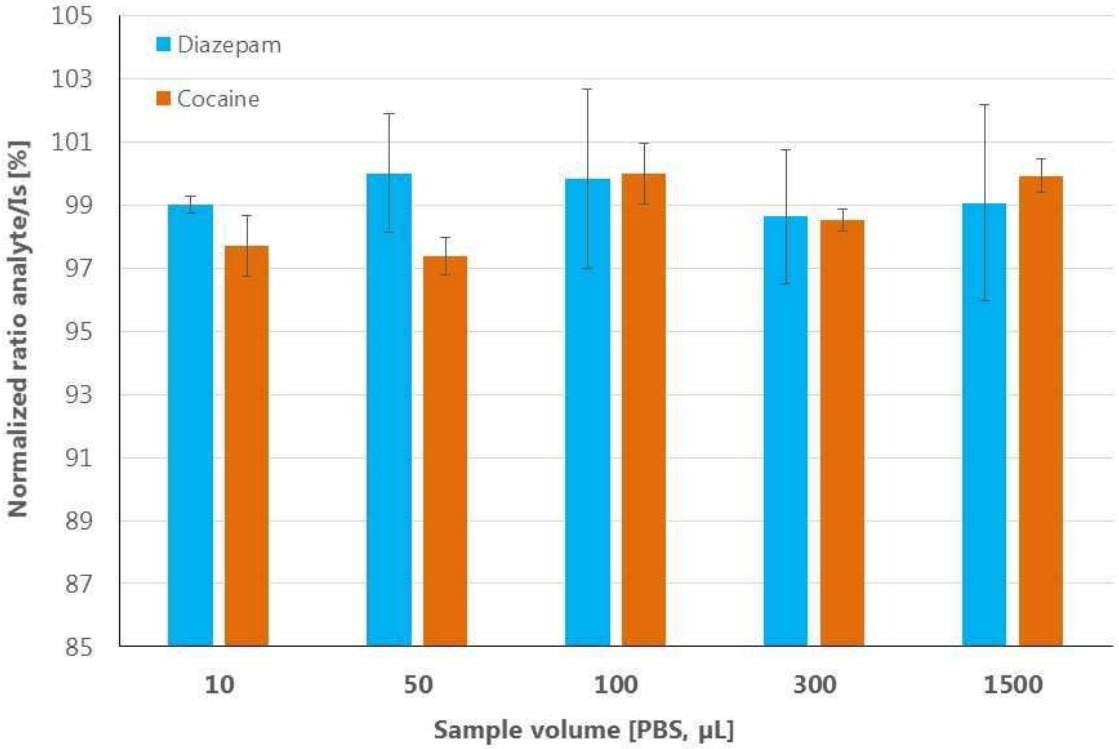

Figure S7 Comparison of analyte-to-internal standard ratios for cocaine and diazepam spiked at 20 ng mL^{-1} in five different volumes of PBS. Results were normalized for easier visualization. Internal standards were spiked at 10 ng mL^{-1}. Bars represent the standard deviation of analyses for three replicates with independent fibers and nano-ESI emitters.

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