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Supporting Information

High-Throughput Monitoring of Cocaine and its Metabolites in Hair Using Microarrays for Mass Spectrometry and MALDI-MS/MS

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min, H_2O/CAN/TFA 15 min and 60 min extraction protocols for 0.5 ng/mg, and H_2O/ACN/TFA 15 min incubation protocol for the level at 5 ng/mg. Error bars represent the standard deviation around the mean of the centroid mass (n=10, 11, or 12). In each histogram, value of signal to noise mean (n=10, 11 or 12) is reported.for six hair extraction protocols.

Figure S5. Full-scan MALDI-FTICR mass spectrum using CHCA matrix. Zoom around the peak at m/z=304.

Figure S6. MALDI mass spectra of hair spiked with cocaine. (A) Full-scan mass spectrum identifying cocaine, with a zoom around the cocaine signal. Asterisks indicate didecyl benzyl dimethyl ammonium C12-BDMA (m/z 304.30), didecyl dimethyl ammonium C10-DMA (m/z 326.30), benzyl tetradecyl dimethyl ammonium C14-BDMA (m/z 332.33) and benzyl acetyl dimethyl ammonium C16-BDMA (m/z 360.36), some quaternary ammonium compounds from toiletry product. Hexagons denote a CHCA matrix ion at *m/z* 304.28. (B) MS/MS spectrum of the precursor m/z 304 including the deuterated cocaine internal standard (m/z 307→ m/z 185). The main cocaine fragment is at *m/z* 182. Asterisks mark fragment ions of C12-BDMA (m/z 212.2 and m/z 91.1), and the hexagon the fragment ion of the CHCA (m/z 215.1).

Table S1. Comparison of the relative standard deviations for three concentrations of cocaine using MALDI regular target plate and MAMS slide.

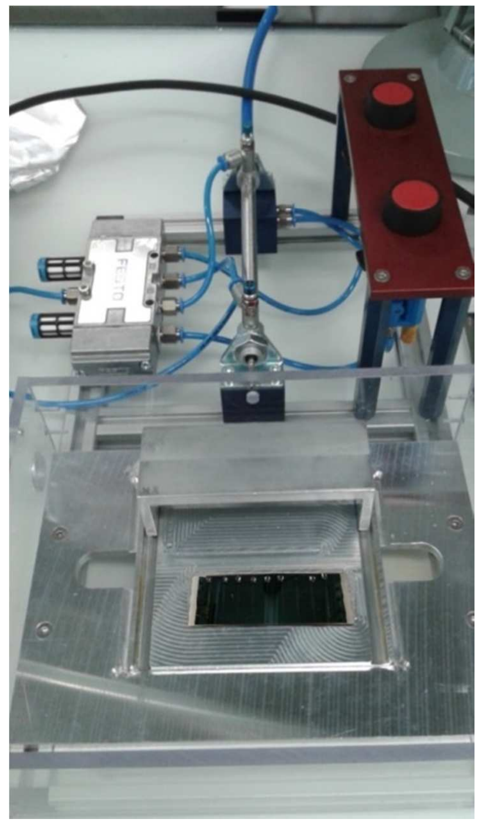
Table S2. Quantitative results for cocaine and its metabolites, obtained from the LC-MS/MS method, applied to four hair samples.

Table S3. Metabolite-parent drug ratios, LC-MS/MS vs MALDI-MS/MS, applied to the four hair samples (A, B, C and D).

SUPPORTING INFORMATION FOR MATERIALS AND METHODS

Figure S1. (A) Images of three MAMS slides fixed with copper adhesive tape on a steel AB SCIEX MALDI target. An additional steel plate was used to adapt the MAMS slides, because silicon MAMS slides are thinner than the microscopic slides usually used, in particular in MALDI imaging. (B) Image of the automated home-made device, used to move drops in the spots of the Si-MAMS. One MAMS slide is put under the metal block. By pressing a red button (in the right top), air pressure (in blue cable) will move a metal bar which in turn, will move the slider in rails flanking the MAMS blade.

(B)



(A)

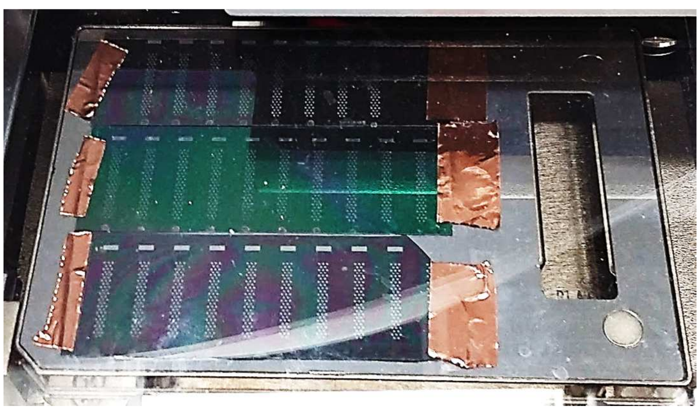


Figure S2. Comparison of the area intensity, resolution and signal-to-noise ratio using CHCA (5 mg/mL), Cl-CCA (5 mg/mL), DHB (15 mg/mL), ATT (10 mg/mL), 3HC (10 mg/mL) and 2H1NA (4 mg/mL) matrices. Analysis of cocaine at 2 µg/mL in reflectron positive ion mode. Parameters used were: laser intensity at 3300 u.a and 1200 shots, 4500 u.a and 300 shots, 4550 u.a and 450 shots, 4500 u.a and 2000 shots, 5130 u.a and 100 shots, 4190 u.a and 400 shots, respectively, for CHCA, Cl-CCA, DHB, ATT, 3HC and 2H1NA (n=4).

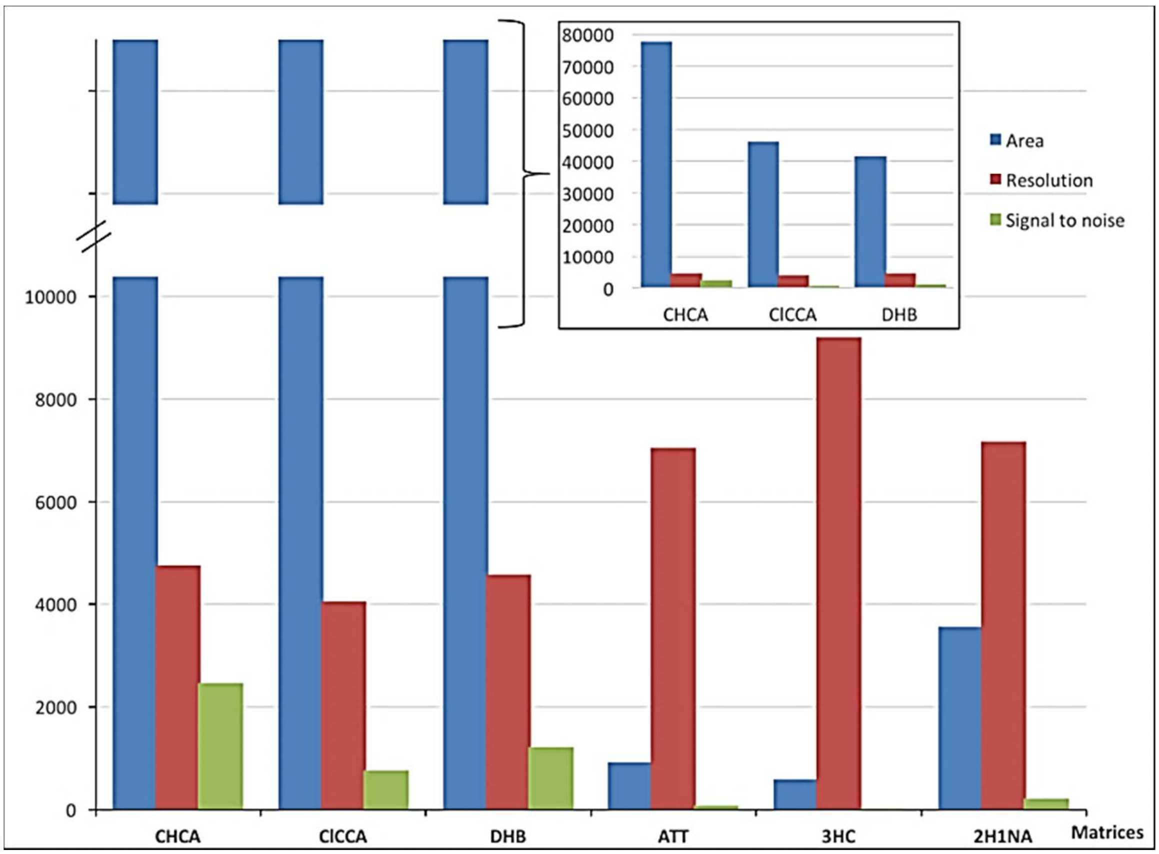


Figure S3. Flow chart of the pre-analytical procedures employed for the hair MAMS-MALDI-TOF analysis.

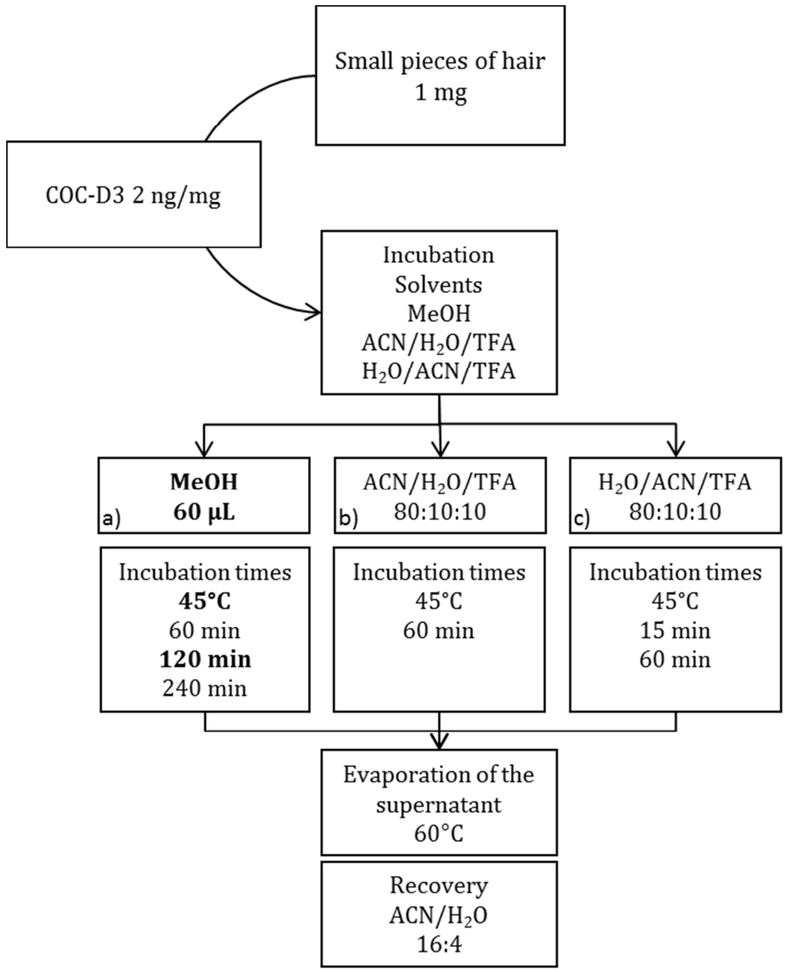
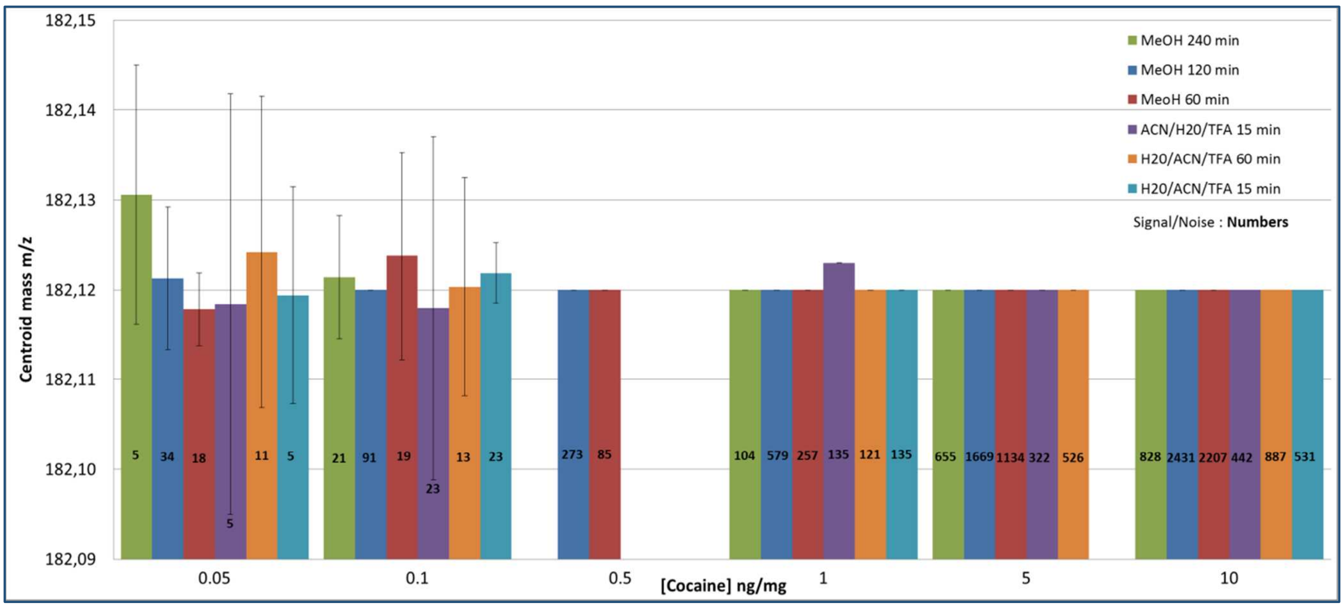


Figure S4. Cocaine quantitation range in 1-mg-hair samples, obtained by MALDI-MS/MS analysis. Histograms showing centroid mass function of concentration levels, from 0.05 to 10 ng/mg, for six hair extraction protocols. MS/MS data of cocaine precursor ion, at m/z=304, are not available for MeOH 240 min, ACN/H_2O/TFA 15 min, H_2O/CAN/TFA 15 min and 60 min extraction protocols for 0.5 ng/mg, and H_2O/ACN/TFA 15 min incubation protocol for the level at 5 ng/mg. Error bars represent the standard deviation around the mean of the centroid mass (n=10, 11, or 12). In each histogram, value of signal to noise mean (n=10, 11 or 12) is reported.



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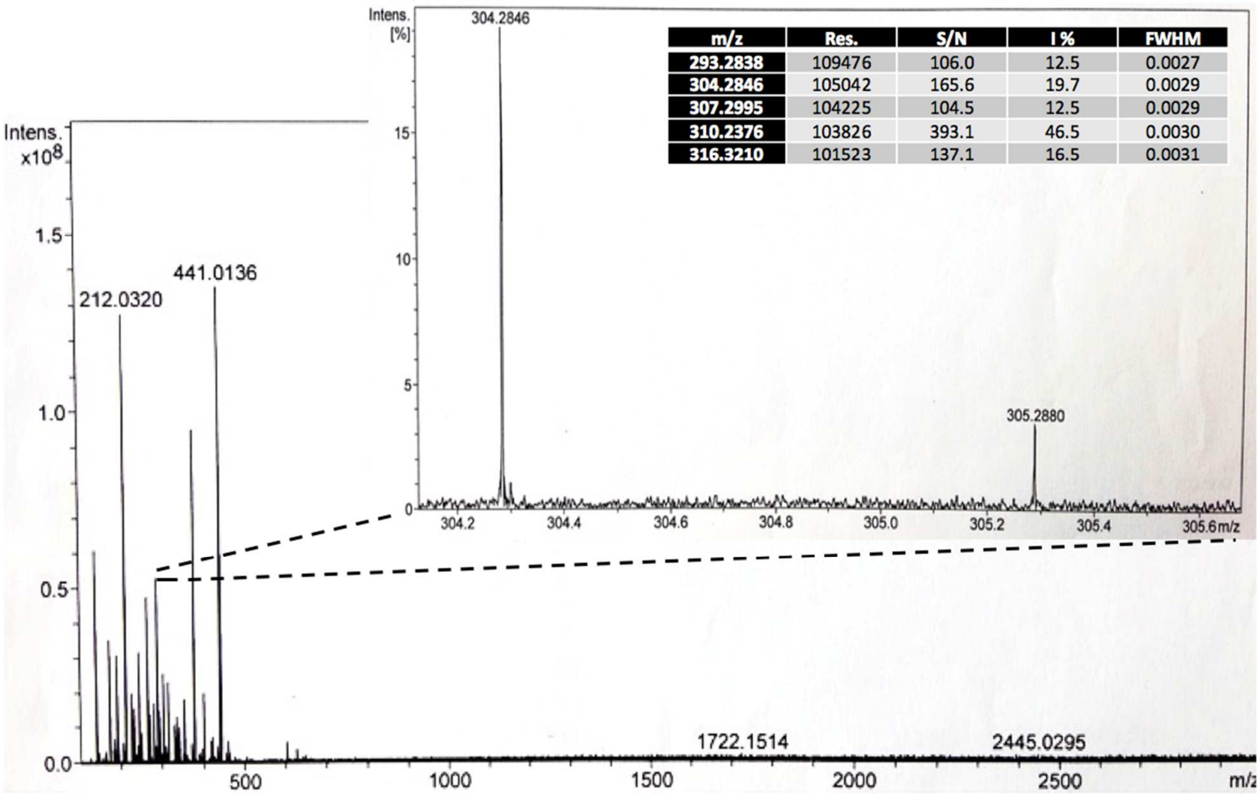

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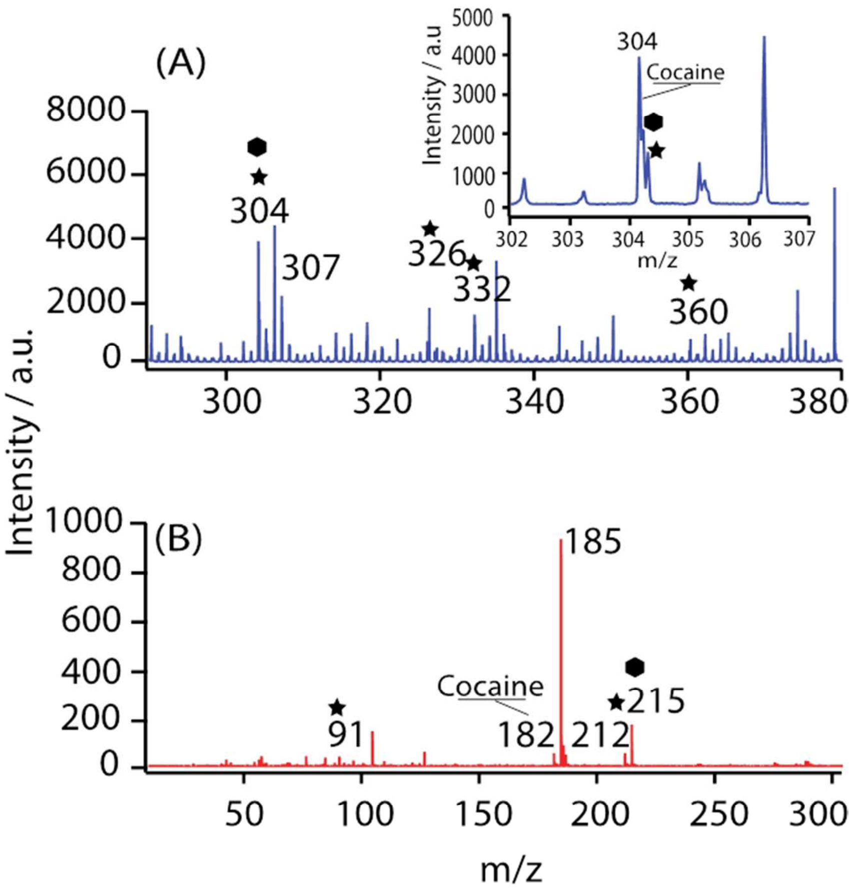

Table S1. Comparison of the relative standard deviations (RSD) for three concentrations of cocaine using MALDI regular target plate and MAMS slide.

Estimated conc. ng/mL	Manual deposition (n=10)						Automated deposition (n=10)		
	Regular MALDI plate			MAMS plate			MAMS plate		
	A_{rel} ($A_{cocaine}/A_{cocaine\text{-}D3}$)	Standard deviation	RSD %	A_{rel} ($A_{cocaine}/A_{cocaine\text{-}D3}$)	Standard deviation	RSD %	A_{rel} ($A_{cocaine}/A_{cocaine\text{-}D3}$)	Standard deviation	RSD %
500	0.98	0.27	27.7	1.64	0.23	14.3	0.78	0.05	6.0
250	0.42	0.07	16.2	0.91	0.10	10.9	0.51	0.03	5.6
50	0.10	0.02	22.7	0.09	0.02	23.7	0.19	0.01	3.2

Table S2. Quantitative results for cocaine and its metabolites, obtained from the LC-MS/MS method, applied to four hair samples (A, B, C and D).

Hair samples	Hair segments	COC ng/mg	BZE ng/mg	CE ng/mg	EME ng/mg
A	0-1 cm	4.46	6.78	0.30	0.31
	1-2 cm	5.61	11.30	0.40	0.25
	2-3 cm	9.83	19.30	0.64	0.27
	3-4 cm	11.20	22.60	0.51	0.24
B	0-1 cm	16.60	7.94	1.27	0.58
	1-2 cm	26.50	11.50	1.59	0.71
	2-3 cm	39.00	16.50	1.71	0.84
	3-4 cm	38.20	15.10	1.56	0.84
C	0-1 cm	4.42	6.01	1.77	0.30
	1-2 cm	4.97	6.73	1.58	0.29
	2-3 cm	4.97	6.99	1.26	0.24
	3-4 cm	6.80	9.98	1.55	0.26
D	0-1 cm	10.2	5.18	1.72	0.326
	1-2 cm	8.43	3.91	1.26	0.156
	2-3 cm	6.91	3.67	0.86	0.071
	3-4 cm	3.65	2.43	0.28	-

- : Quantity of drug below the limit of the quantitation (LOQ)

Table S3. Metabolite-parent drug ratios, LC-MS/MS vs MALDI-MS/MS, applied to the four hair samples (A, B, C and D).

Hair sample	Hair segment	BZE/COC ratio		CE/COC ratio	
		LC-MS/MS	MALDI-MS/MS	LC-MS/MS	MALDI-MS/MS
A	0-1 cm	1.3	1.8	0.07	0.10
	1-2 cm	1.7	2.1	0.08	0.10
	2-3 cm	1.7	2.0	0.07	0.10
	3-4 cm	1.7	1.9	0.05	0.06
B	0-1 cm	0.4	0.5	0.09	0.32
	1-2 cm	0.4	0.5	0.07	0.26
	2-3 cm	0.4	0.4	0.05	0.21
	3-4 cm	0.4	0.4	0.05	0.20
C	0-1 cm	1.2	1.2	0.46	0.47
	1-2 cm	1.2	1.1	0.36	0.74
	2-3 cm	1.2	1.2	0.29	0.65
	3-4 cm	1.3	1.1	0.26	0.31
D	0-1 cm	0.4	0.3	0.21	0.12
	1-2 cm	0.4	0.3	0.19	0.10
	2-3 cm	0.5	0.3	0.16	0.07
	3-4 cm	0.6	0.3	0.12	0.05