$Electronic Supplementary Information

Matrix-assisted laser desorption/ionization mass spectrometry analysis of dimethyl arginine isomers from urine

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Supplementary Table 1. Calibration statistics of ADMA/SDMA common fragment/product ions analyzed using MALDI-TOF MS

Analyte	Ion (*m/z*)	Calibration range (in µM)	Calibration Equation; R^2	QC samples (in µM)	% Recovery (% RSD)
SDMA	203.1508	0.5-10	y=0.029x-0.012; 0.98	2	74 (10.2)
				8	99 (19.0)
	158.1265	0.75-10	y=0.025x+0.0009; 0.99	2	97 (15.2)
				8	120 (13.6)
	116.0589	0.75-10	y=0.049x-0.0046; 0.99	2	50 (13.9)
				8	76 (4.5)
	88.0873	1-10	y=0.013x+0.0060; 0.99	2	86 (13.5)
				8	57 (15.2)
	70.0756	1-10	y=0.026x+0.0010; 0.99	2	53 (13.7)
				8	89 (12.3)
ADMA	203.1508	0.5-10	y=0.050x+0.032; 0.99	2	105 (10.4)
				8	120 (16.7)
	158.1293	0.75-10	y=0.028x+0.031; 0.95	2	92 (17.0)
				8	105 (17.8)
	116.0563	0.75-10	y=0.053x+0.035; 0.95	2	96 (17.6)
				8	114 (14.8)
	88.0930	1-10	y=0.024x+0.071; 0.98	2	108 (10.8)
				8	136 (15.8)
	70.0771	1-10	y=0.023x+0.054; 0.98	2	147 (22.6)
				8	153 (17.4)

Supplementary Table 2. Calibration statistics for unique fragment ions of ADMA (*m/z* 46.0670) and SDMA (*m/z* 172.1090) covering concentrations relevant in the abnormal range demonstrating the applicability of the method

Analyte	Calibration range (in µM)	Equation; R^2	QC sample (µM)	% Recovery	Intra-assay variation (%RSD)	Inter-assay variation (%RSD)
ADMA	10-100	y=0.001x+0.008; 0.94	25	91	6.9	7.4
			55	92	12.9	5.9
			75	102	12.7	9.5
SDMA	10-100	y=0.105x+1.096; 0.92	25	95	9.9	3.1
			55	88	9.0	2.6
			75	111	7.1	6.4

Supplementary Table 3. Mass accuracy (MA) parameters for ADMA and SDMA across three days. The obtained MA parameters are calculated according to the cited reference.[1]

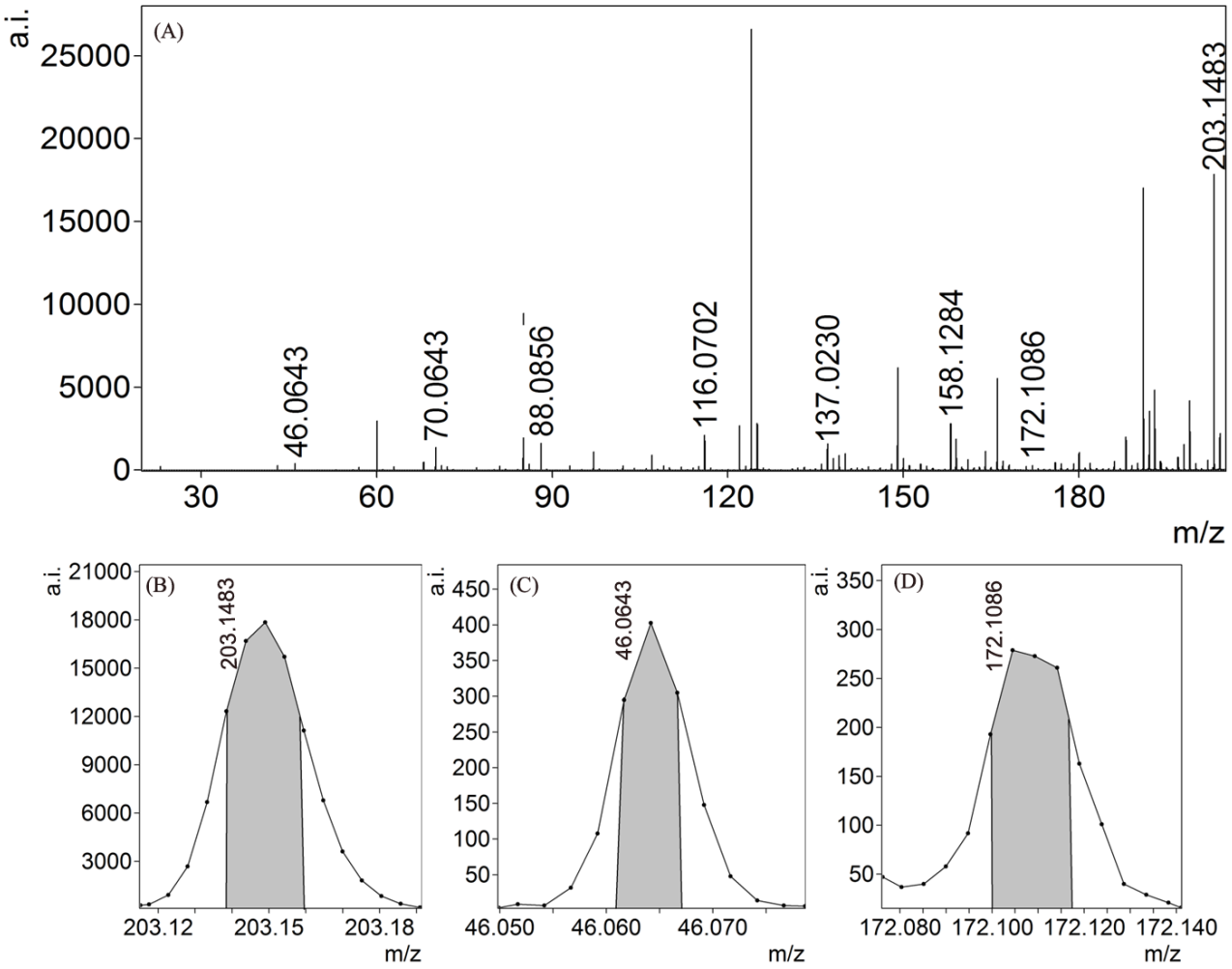
Features	ADMA (*m/z* 46)	SDMA (*m/z* 172)
Calibrators in the lower concentration range (in µM)	1.00 1.50 2.00 2.50 5.00 7.50 10.00	0.50 0.60 0.75 0.80 1.00 2.50 5.00 7.50 10.00
Calibrators in the higher concentration range (in µM)	10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00	10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00
QCs in solvent system (in µM)	1.25 4.00 8.00 25.00 55.00 75.00	1.25 4.00 8.00 25.00 55.00 75.00
Analyte concentration determined from pooled urine (in µM)	5.8	14.1
QCs spiked in pooled urine (in µM)	1.25 4.00	1.25 4.00
Concentrations of unknowns determined from urine samples of 11 individuals (in µM)	4.04 1.60 2.83 7.86 1.80 4.18 1.80 1.90 1.73 2.57	5.65 2.29 3.29 3.68 2.92 7.16 2.70 2.76 2.47 6.03

	1.90	2.83
MA_{mean} (ppm)	9.5	9.5
MAP_{mean} (ppm)	2.6	5.6
MAV (ppm)	5	5
$MA\text{-}AC_{intra\text{-}assay}$ (ppm)	1.8 to 17.3	-7.3 to 26.2
$MA\text{-}AC_{standard}$ (ppm)	4.5 to 14.5	4.5 to 14.5
± $MEW_{narrowest}$ (ppm)	19.9	31.8
± $MEW_{broadest}$ (ppm)	114.5	114.5
± $MEW_{standard}$ (ppm)	14.5	14.5
Resolution at *m/z*	10,000	10,000
m/z	46.0651	172.1081

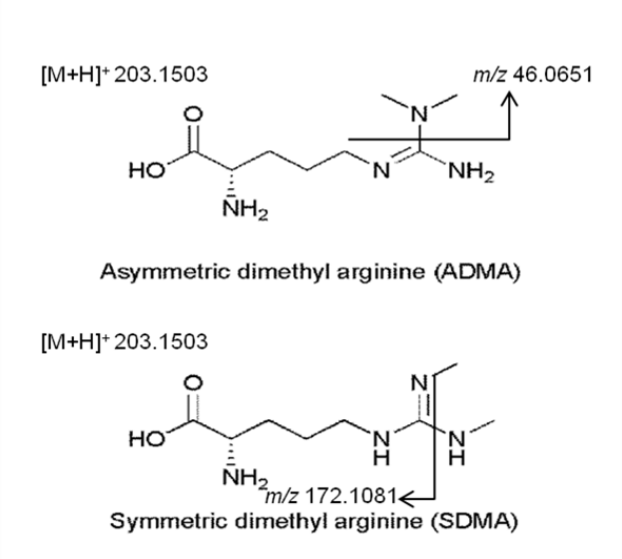
Note: MA_{mean} – Mean of mass accuracies observed, MAP_{mean} – mass accuracy precision for mean of mass accuracy, MAV – maximal instrumental variability, $MA\text{-}AC_{intra\text{-}assay}$ – mass accuracy acceptable criterion for intra-assay, $MA\text{-}AC_{standard}$ – mass accuracy acceptable criterion for standard, $MEW_{narrowest}$ – narrowest mass extraction window, $MEW_{broadest}$ - broadest mass extraction window possible, $MEW_{standard}$ – standard mass extraction window.[1]

Reference

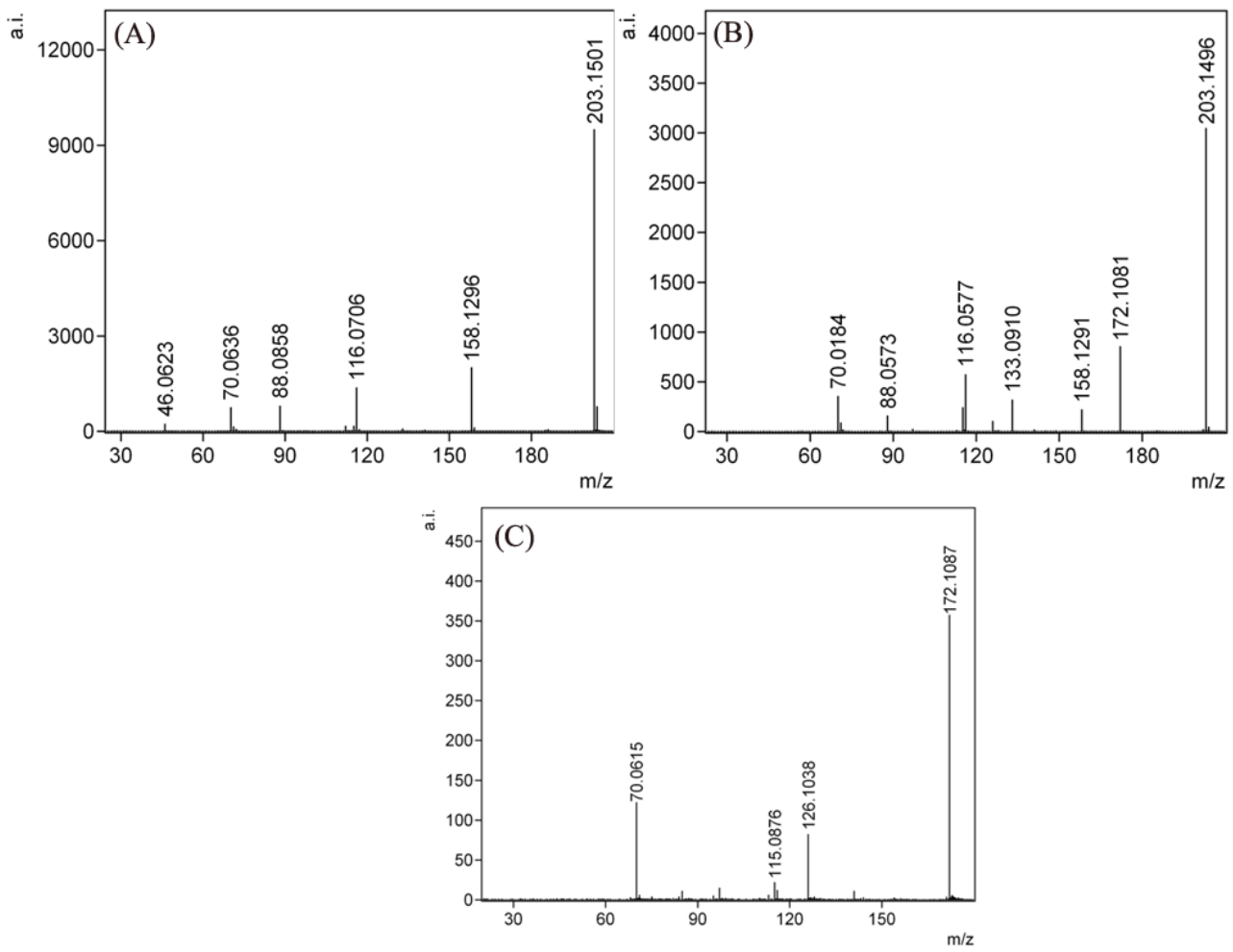
1. B. Rochat, E. Kottelat and J. McMullen, *Bioanalysis*, 2012, 4, 2939-2958.



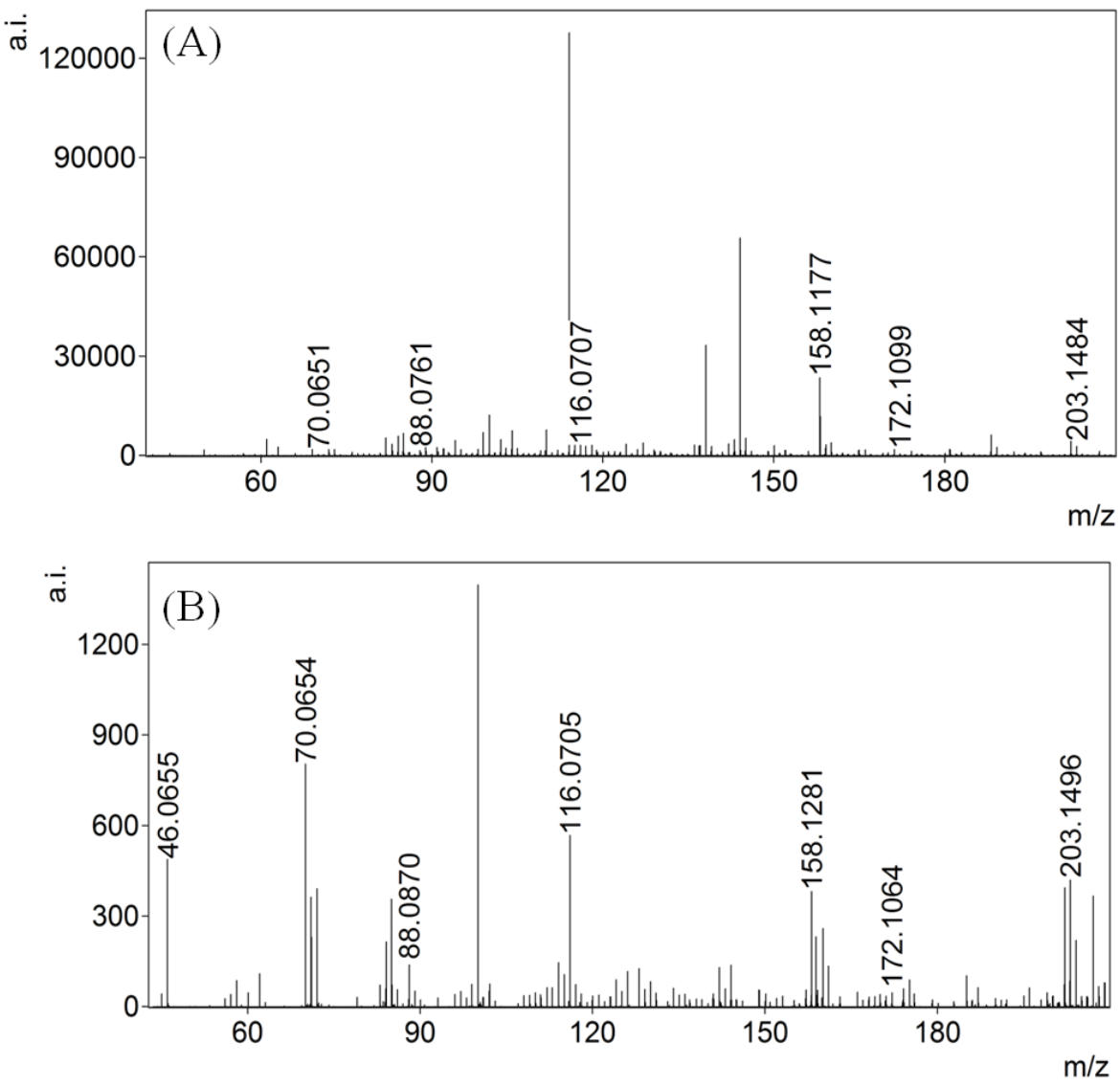
Supplementary figure 1. (A) MALDI MS of a 5 µM mixture of ADMA and SDMA in the *m/z* range 20-210 depicting the ions of ADMA and SDMA along with the matrix peaks. (B-D) represents the (B) protonated adduct ion peak of ADMA/SDMA at *m/z* 203.1483, (C) product ion of ADMA at *m/z* 46.0643 and (D) product ion of SDMA at *m/z* 172.1086 respectively with highlighted area in gray shade representing area under curve considered for quantitation.



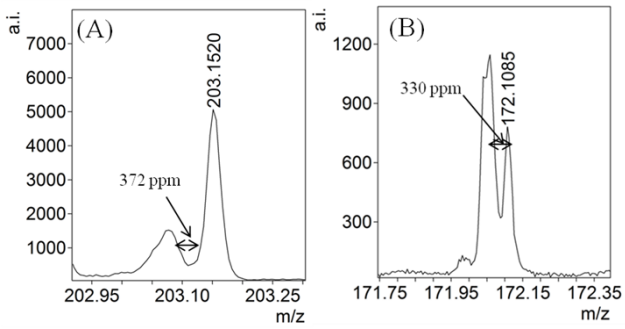
Supplementary figure 2. Structures of ADMA and SDMA along with their respective unique fragment ions formed in the MS or MS/MS mode. The exact masses of protonated adducts are indicated by $[M+H]^+$ notations and exact masses of the unique product ion are indicated by *m/z* notation.



Supplementary figure 3. MALDI MS/MS of (A) ADMA and (B) SDMA at *m/z* 203.1501 and *m/z* 203.1496, respectively depicting protonated adduct ion $[M+H]^+$ as reported in previous literature. (C) Pseudo-MS^3 for unique product ion of SDMA at *m/z* 172.1087.



Supplementary figure 4. (A) Direct injection ESI MS spectrum of urine sample in the *m/z* range 40-210. Ions at *m/z* 203.1484, 158.1177, 116.0707, 88.0761 and 70.0651 indicate the presence of endogenous DMA isomers; unique product ion observed at *m/z* 172.1099 is indicative of SDMA. Unique product ion peak of ADMA at *m/z* 46 was not observed in ESI MS from urine samples. (B) Unique product ion peaks observed at *m/z* 46.0655 and 172.1064 upon CID fragmentation of the precursor ion, *m/z* 203 (supplementary figure 5A) confirms the presence of endogenous ADMA and SDMA. Fragmentation observed in ESI-MS and MS/MS are in agreement with previously reported data (P. M. Gehrig, P. E. Hunziker, S. Zahariev and S. Pongor, *J. Am. Soc. Mass. Spectrom.* , 2004, 15, 142-149).



Supplementary figure 5. Zoomed regions in the *m/z* range of the analytes of interest subjected to MALDI MS from urine samples depicting clear resolution from potential interferences. The value in ppm illustrated the difference between the adjacent peak and analyte of interest for (A) ADMA /SDMA protonated adduct ion $[M+H]^+$ peak at *m/z* 203.1520 and (B) SDMA product ion at *m/z* 172.1085.