ELECTRONIC SUPPORTING INFORMATION.

COMPARISON BETWEEN ONE AND TWO-DIMENSIONAL LIQUID CHROMATOGRAPHIC APPROACHES FOR THE DETERMINATION OF PLASMATIC STROKE BIOMARKERS BY ISOTOPE DILUTION AND TANDEM MASS SPECTROMETRY

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Number of pages 10
Number of Tables: 9
Number of Figure: 7

Figure S1. Comparison of the theoretical and experimental isotopologue distributions obtained by LC-MSMS using the SRM mode for the natural abundance and labelled analogues of a) L-Arginine, b) ADMA and c) SDMA. The theoretical isotopic distributions were obtained according to reference [**]. The uncertainty of the experimental values represents the 1s standard deviation of n = 6 LC–MS/MS injections.

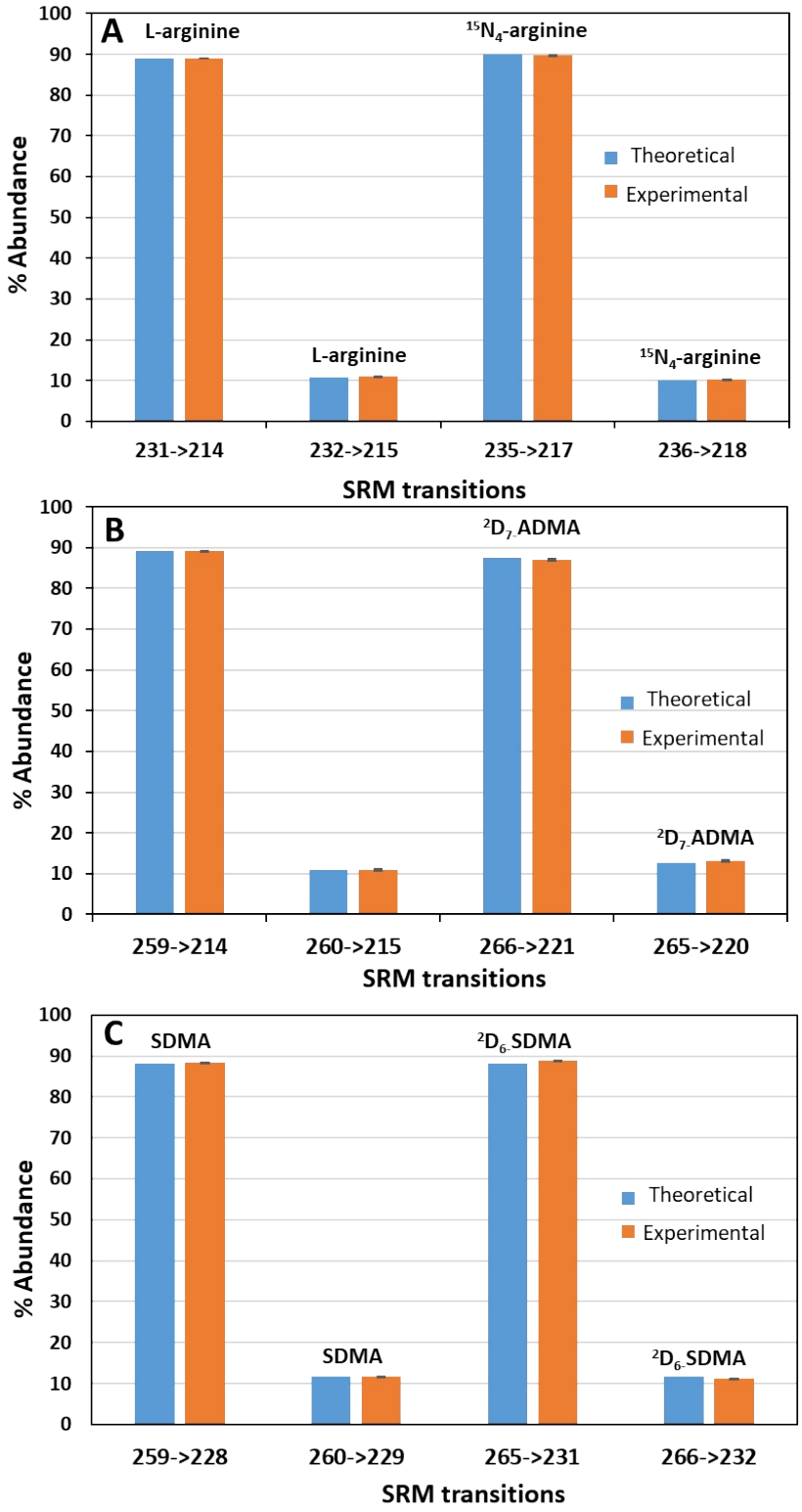


Figure S2. 1D-HPLC-UV chromatogram of a standard solution containing 400 µg g^{-1} of L-Arg, 300 µg g^{-1} of ADMA and 200 µg g^{-1} of SDMA using 1% HFBA in water and acetonitrile (mobile phases A and B, respectively).

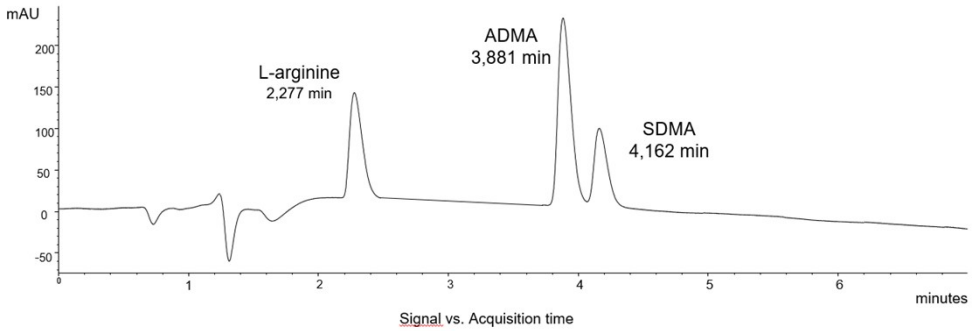


Figure S3. Peak area for L-Arg, ADMA and SDMA in a pooled plasma by 2D-HPLC-MS/MS obtained at injection volumes from 0.5 to 5µL.

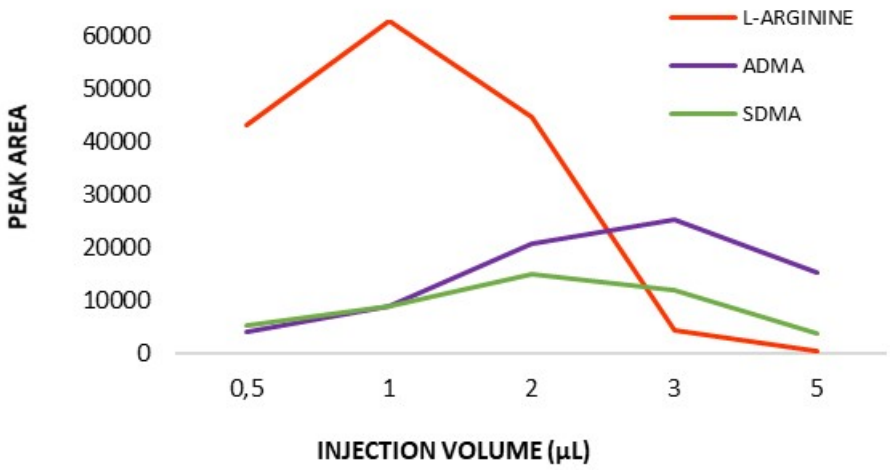


Table S1. Added concentration, experimental concentration, and recovery for L-Arg obtained by 2D-HPLC-MS/MS in a pooled plasma fortified with known amounts of the natural abundance L-Arg at 3 concentration levels obtained in two different measurement days. Uncertainty of the individual recovery values is expressed as the standard deviation of n=3 independent injections into the 2D-HPLC-MS/MS.

L-Arginine by 2D-HPLC-MS/MS					
Level	**Measurement Day**	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
1	1	1	7.89	7.59 ± 0.04	96.23 ± 0.47
		2	7.76	7.51 ± 0.10	96.74 ± 1.25
		3	7.89	7.78 ± 0.13	98.58 ± 1.59
	2	4	7.74	7.46 ± 0.17	96.46 ± 2.26
		5	8.53	8.11 ± 0.27	95.14 ± 3.17
		6	8.09	7.67 ± 0.06	94.84 ± 0.79
2	1	1	15.81	15.78 ± 0.59	99.81 ± 3.75
		2	15.60	15.46 ± 0.13	99.12 ± 0.83
		3	15.66	15.52 ± 0.21	99.08 ± 1.33
	2	4	15.54	15.08 ± 0.31	97.02 ± 2.00
		5	15.83	15.13 ± 0.22	95.56 ± 1.40
		6	15.87	14.95 ± 0.24	94.23 ± 1.54
3	1	1	32.05	31.36 ± 0.21	97.84 ± 0.65
		2	31.91	30.37 ± 0.25	98.31 ± 0.79
		3	31.23	30.67 ± 0.05	98.22 ± 0.17
	2	4	31.46	29.99 ± 0.52	95.33 ± 1.64
		5	31.51	29.36 ± 0.18	93.16 ± 0.58
		6	31.52	29.66 ± 1.14	94.09 ± 3.63

Table S2. Added concentration, experimental concentration, and recovery for ADMA obtained by 2D-HPLC-MS/MS in a pooled plasma fortified with known amounts of the natural abundance L-Arg at 3 concentration levels obtained in two different measurement days. Uncertainty of the individual recovery values is expressed as the standard deviation of n=3 independent injections into the 2D-HPLC-MS/MS.

ADMA by 2D-HPLC-MS/MS					
Level	**Measurement Day**	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
1	1	1	0.18	0.20 ± 0.03	110.63 ± 18.20
		2	0.18	0.27 ± 0.01	153.58 ± 4.85
		3	0.18	0.22 ± 0.00	120.28 ± 1.72
	2	4	0.17	0.47 ± 0.00	268.33 ± 2.28
		5	0.19	0.50 ± 0.01	257.98 ± 5.22
		6	0.18	0.48 ± 0.01	260.60 ± 3.30
2	1	1	0.36	0.37 ± 0.06	103.34 ± 17.85
		2	0.36	0.50 ± 0.03	140.71 ± 7.37
		3	0.36	0.55 ± 0.02	154.09 ± 4.56
	2	4	0.35	0.77 ± 0.02	220.22 ± 4.55
		5	0.36	0.98 ± 0.01	218.82 ± 4.15
		6	0.36	0.78 ± 0.04	218.40 ± 9.85
3	1	1	0.73	0.96 ± 0.02	130.56 ± 2.47
		2	0.73	0.98 ± 0.04	134.61 ± 4.89
		3	0.71	0.68 ± 0.01	95.92 ± 2.09
	2	4	0.71	1.45 ± 0.08	203.92 ± 11.47
		5	0.71	1.49 ± 0.05	210.13 ± 7.69
		6	0.71	1.52 ± 0.08	213.29 ± 10.98

Table S3. Added concentration, experimental concentration, and recovery for SDMA obtained by 2D-HPLC-MS/MS in a pooled plasma fortified with known amounts of the natural abundance L-Arg at 3 concentration levels obtained in two different measurement days. Uncertainty of the individual recovery values is expressed as the standard deviation of n=3 independent injections into the 2D-HPLC-MS/MS.

SDMA by 2D-HPLC MS/MS					
Level	**Measurement Day**	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
1	1	1	0.65	1.12 ± 0.04	170.51 ± 6.03
		2	0.64	1.13 ± 0.02	174.78 ± 2.68
		3	0.65	1.09 ± 0.03	166.45 ± 2.23
	2	4	0.66	1.22 ± 0.02	184.46 ± 2.58
		5	0.73	1.34 ± 0.02	185.08 ± 3.06
		6	0.69	1.24 ± 0.03	180.44 ± 4.19
Level 2	1	1	1.31	2.17 ± 0.06	165.67 ± 4.90
		2	1.29	2.22 ± 0.01	171.38 ± 0.47
		3	1.30	2.24 ± 0.03	172.35 ± 2.01
	2	4	1.32	2.17 ± 0.03	163.63 ± 1.98
		5	1.35	2.28 ± 0.02	168.92 ± 1.45
		6	1.35	2.31 ± 0.03	170.86 ± 2.33
Level 3	1	1	2.66	4.33 ± 0.03	162.73 ± 0.96
		2	2.65	4.33 ± 0.03	163.56 ± 1.08
		3	2.59	3.73 ± 0.05	143.98 ± 1.94
	2	4	2.68	4.57 ± 0.04	170.69 ± 1.59
		5	2.68	4.67 ± 0.09	173.85 ± 3.50
		6	2.68	4.78 ± 0.19	177.95 ± 6.91

Figure S4. Comparison of the peak area obtained for the most abundant SRM transition of natural abundance L-Arginine, ADMA and SDMA 1D-HPLC-MS/MS and 2D-HPLC-MS/MS.

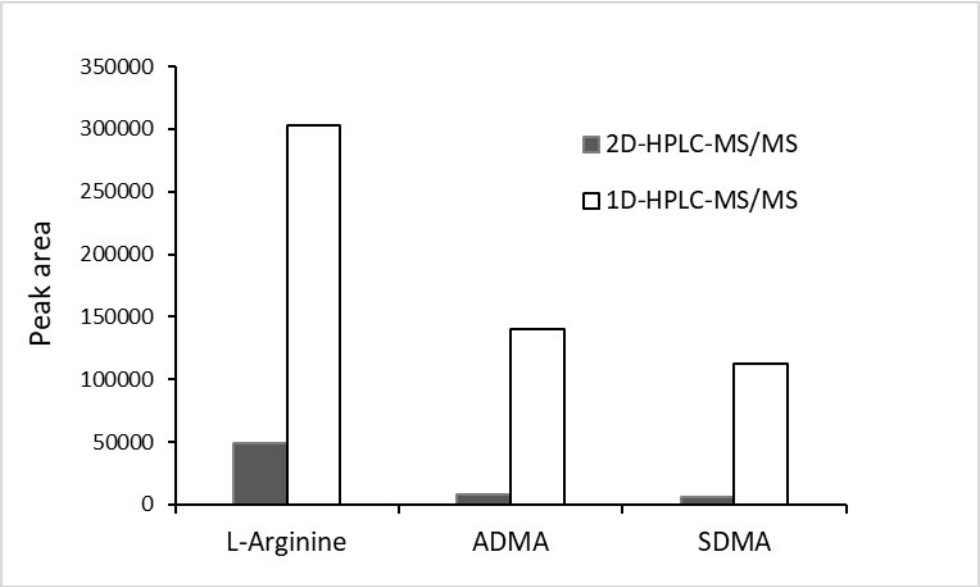


Table S4. Added concentration, experimental concentration and recovery for L-Arg in a pooled plasma enriched with a known amount of the natural abundance analytes to achieve 3 concentration levels measured by 1D-HPLC-MS/MS. This recovery experiment was carried out in four days and n=3 independent replicates were measured in triplicate. Uncertainty of the values correspond to the standard deviation obtained from n=3 independent injections.

L-Arginine by 1D-HPLC-MS/MS					
Level	**Measurement Day**	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
1	1	1	7.92	8.04 ± 0.05	101.56 ± 0.69
		2	8.15	8.33 ± 0.04	102.19 ± 0.46
		3	7.72	7.81 ± 0.04	101.26 ± 0.56
	2	4	8.34	8.47 ± 0.03	101.55 ± 0.31
		5	7.31	8.41 ± 0.04	101.31 ± 0.55
		6	7.29	7.40 ± 0.04	101.51 ± 0.51
	3	7	8.52	8.72 ± 0.03	101.31 ± 0.33
		8	8.18	8.26 ± 0.03	100.97 ± 0.32
		9	8.50	8.87 ± 0.04	104.37 ± 0.47
	4	10	8.32	7.87 ± 0.14	94.54 ±1.69
		11	8.34	7.98 ± 0.11	95.69 ± 1.37
		12	8.22	8.73 ± 0.06	106.23 ± 0.70
2	1	1	16.06	16.44 ± 0.09	102.32 ± 0.53
		2	15.69	15.70 ± 0.64	100.09 ± 4.10
		3	16.28	16.63 ± 0.09	102.17 ± 0.53
	2	4	16.92	16.71 ± 0.03	98.75 ± 0.20
		5	16.41	16.46 ± 0.15	100.27 ± 0.89
		6	16.78	16.77 ± 0.02	99.94 ± 0.13
	3	7	16.06	16.13 ± 0.08	100.44 ± 0.51
		8	14.25	15.49 ± 1.75	108.75 ± 12.29
		9	15.72	16.08 ± 0.06	102.25 ± 0.36
	4	10	16.50	16.74 ± 0.07	101.42 ± 0.43
		11	14.22	18.30 ± 0.69	128.62 ± 4.83
		12	15.96	16.94 ± 0.07	106.11 ± 0.44
3	1	1	32.03	32.62 ± 0.14	101.82 ± 0.45
		2	32.30	33.05 ± 0.07	102.32 ± 0.22
		3	32.20	33.00 ± 0.15	102.50 ± 0.45
	2	4	32.23	32.10 ± 0.04	99.61 ± 0.13
		5	32.25	33.14 ± 0.08	102.75 ± 0.25
		6	31.99	32.68 ± 0.04	102.17 ± 0.13

	3	7	34.07	36.30 ± 0.05	106.55 ± 0.14
		8	32.95	34.78 ± 0.29	105.55 ± 0.88
		9	31.86	33.28 ± 0.04	104.47 ± 0.14
	4	10	33.11	34.52 ± 0.11	104.26 ± 0.34
		11	32.48	34.18 ± 0.06	105.23 ± 0.19
		12	33.24	34.40 ± 0.11	103.51 ± 0.34

Table S5. Added concentration, experimental concentration and recovery for ADMA in a pooled plasma enriched with a known amount of the natural abundance analytes to achieve 3 concentration levels measured by 1D-HPLC-MS/MS. This recovery experiment was carried out in four days and n=3 independent replicates were measured in triplicate. Uncertainty of the values correspond to the standard deviation obtained from n=3 independent injections.

ADMA by 1D-HPLC-MS/MS					
Level	**Measurement Day**	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
1	1	1	0.19	0.20 ± 0.00	104.16 ± 1.16
		2	0.20	0.21 ± 0.01	105.84 ± 3.24
		3	0.19	0.20 ± 0.00	104.54 ± 1.59
	2	4	0.19	0.20 ± 0.00	104.31 ± 0.54
		5	0.17	0.17 ± 0.00	102.71 ± 1.02
		6	0.17	0.17 ± 0.00	103.94 ± 0.78
	3	7	0.20	0.21 ± 0.01	103.24 ± 4.01
		8	0.19	0.19 ± 0.00	98.32 ± 1.41
		9	0.20	0.21 ± 0.00	105.91 ± 1.72
	4	10	0.20	0.19 ± 0.00	96.62 ± 1.90
		11	0.20	0.19 ± 0.00	96.95 ± 1.85
		12	0.20	0.21 ± 0.00	109.17 ± 0.71
2	1	1	0.39	0.41 ± 0.00	105.41 ± 1.03
		2	0.38	0.40 ± 0.00	105.25 ± 0.98
		3	0.39	0.42 ± 0.01	105.11 ± 2.14
	2	4	0.39	0.40 ± 0.00	102.73 ± 1.24
		5	0.38	0.40 ± 0.01	105.14 ± 1.54
		6	0.39	0.40 ± 0.00	103.90 ± 1.02
	3	7	0.38	0.39 ± 0.00	102.28 ± 1.15
		8	0.34	0.36 ± 0.00	105.02 ± 0.04
		9	0.37	0.39 ± 0.00	105.11 ± 1.13
	4	10	0.39	0.42 ± 0.00	106.72 ± 0.68
		11	0.34	0.44 ± 0.00	130.07 ± 0.56
		12	0.38	0.42 ± 0.00	110.35 ± 0.41
3	1	1	0.78	0.80 ± 0.00	103.52 ± 0.30
		2	0.78	0.82 ± 0.00	104.43 ± 0.47
		3	0.78	0.82 ± 0.01	104.87 ± 1.64
	2	4	0.74	0.78 ± 0.01	105.93 ± 1.10
		5	0.74	0.80 ± 0.01	108.16 ± 0.69
		6	0.73	0.79 ± 0.01	107.57 ± 1.06
	3	7	0.81	0.84 ± 0.00	104.24 ± 0.36

		8	0.78	0.83 ± 0.00	105.54 ± 0.45
		9	0.76	0.79 ± 0.00	104.67 ± 0.26
	4	10	0.79	0.84 ± 0.01	107.08 ± 0.67
		11	0.77	0.80 ± 0.01	103.36 ± 0.73
		12	0.79	0.83 ± 0.00	105.05 ± 0.42

Table S6. Added concentration, experimental concentration and recovery for SDMA in a pooled plasma enriched with a known amount of the natural abundance analytes to achieve 3 concentration levels measured by 1D-HPLC-MS/MS. This recovery experiment was carried out in four days and n=3 independent replicates were measured in triplicate. Uncertainty of the values correspond to the standard deviation obtained from n=3 independent injections.

SDMA by 1D-HPLC- MS/MS					
	Measurement Day	**Replicate**	**Added Concentration (µg g^{-1})**	**Found concentration (µg g^{-1})**	**% Recovery**
Level 1	1	1	0.64	0.63 ± 0.01	98.45 ± 1.36
		2	0.66	0.66 ± 0.00	99.62 ± 0.35
		3	0.62	0.63 ± 0.00	100.83 ± 0.55
	2	4	0.61	0.60 ± 0.00	98.74 ± 0.21
		5	0.54	0.52 ± 0.00	96.93 ± 0.92
		6	0.53	0.53 ± 0.01	99.01 ± 1.11
	3	7	0.66	0.66 ± 0.00	99.39 ± 0.73
		8	0.63	0.62 ± 0.00	98.44 ± 0.56
		9	0.66	0.66 ± 0.00	100.60 ± 0.75
	4	10	0.64	0.64 ± 0.00	98.92 ± 0.21
		11	0.64	0.64 ± 0.00	99.63 ± 0.76
		12	0.64	0.65 ± 0.00	102.41 ± 0.60
Level 2	1	1	1.30	1.27 ± 0.00	98.12 ± 0.10
		2	1.27	1.26 ± 0.01	99.19 ± 0.62
		3	1.32	1.29 ± 0.00	98.28 ± 0.18
	2	4	1.24	1.30 ± 0.01	104.60 ± 0.42
		5	1.20	1.27 ± 0.01	105.32 ± 0.43
		6	1.23	1.29 ± 0.01	104.59 ± 0.78
	3	7	1.24	1.26 ± 0.00	101.77 ± 0.33
		8	1.10	1.14 ± 0.00	103.38 ± 0.15
		9	1.22	1.23 ± 0.01	101.38 ± 0.60
	4	10	1.28	1.26 ± 0.01	98.97 ± 0.58
		11	1.10	1.37 ± 0.01	124.98 ± 0.50
		12	1.23	1.27 ± 0.01	102.80 ± 0.51
Level 3	1	1	2.59	2.48 ± 0.02	95.97 ± 0.59
		2	2.61	2.37 ± 0.01	90.73 ± 0.27
		3	2.60	2.38 ± 0.01	91.36 ± 0.41
	2	4	2.36	2.27 ± 0.01	96.07 ± 0.43
		5	2.36	2.17 ± 0.02	91.94 ± 0.72
		6	2.34	2.14 ± 0.01	91.38 ± 0.22
	3	7	2.63	2.52 ± 0.01	95.84 ± 0.33
		8	2.55	2.28 ± 0.01	89.49 ± 0.41
		9	2.46	2.21 ± 0.01	89.93 ± 0.22

	4	10 11 12	2.56 2.51 2.57	2.54 ± 0.02 2.30 ± 0.01 2.31 ± 0.00	99.17 ± 0.87 91.62 ± 0.33 90.05 ± 0.19

Table S7. Concentration (µg g^{-1}) of natural and labelled compounds for linearity assessment. Uncertainty of the values correspond to the standard deviation of the concentration obtained for 3 replicates.

Level	L-arginine (µg g^{-1})	$^{15}N_4$-arginine (µg)	ADMA (µg g^{-1})	$^{2}H_7$-ADMA (µg)	SDMA (µg g^{-1})	$^{2}H_6$-SDMA (µg)
N1	1.33 ± 0.00	3.55	0.03 ± 0.00	0.19	0.05 ± 0.00	0.33
N2	3.55 ± 0.01	3.45	0.08 ± 0.00	0.19	0.14 ± 0.00	0.32
N3	7.26 ± 0.05	3.53	0.17 ± 0.00	0.19	0.27 ± 0.00	0.33
N4	15.74 ± 0.03	3.49	0.37 ± 0.00	0.19	0.88 ± 0.00	0.32
N5	23.98 ± 0.03	3.57	0.57 ± 0.00	0.19	1.56 ± 0.01	0.33
N6	39.37 ± 0.04	3.61	0.95 ± 0.01	0.20	2.54 ± 0.01	0.34
N7	73.57 ± 0.12	3.65	1.75 ± 0.01	0.20	4.81 ± 0.01	0.34

Table S8. Number of injections, concentration (ng g^{-1}), LOD and LOQ of L-Arg, ADMA and SDMA obtained from the measurement of 6 replicates of blank samples. Uncertainty of the values correspond to the standard deviation of the concentration obtained for the replicates indicated.

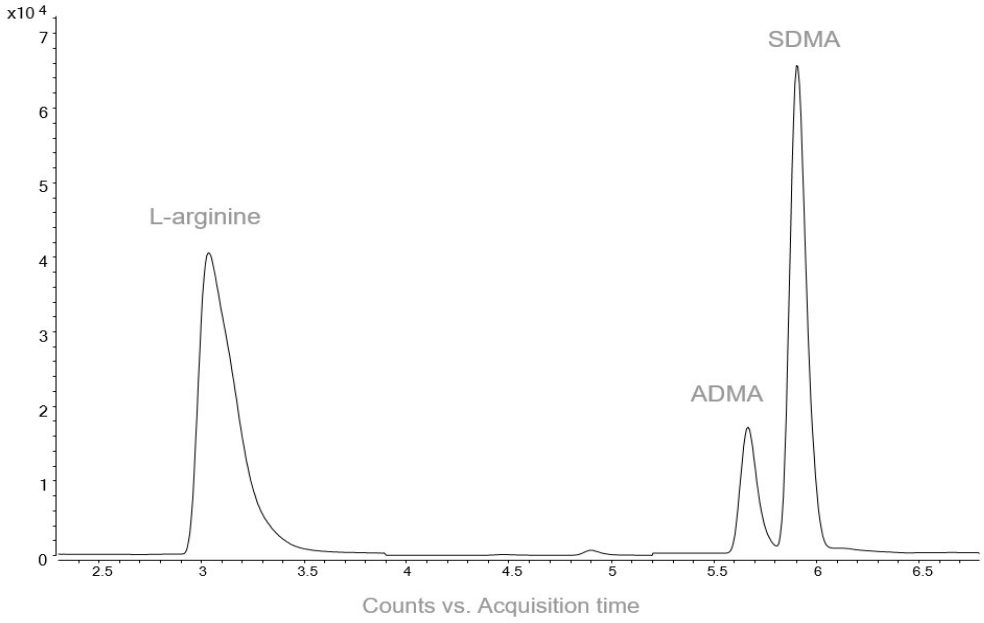
Replicates	Injections	Concentration (ng g^{-1})		
		L-Arginine	ADMA	SDMA
1	10	37.86 ± 1.03	0.07 ± 0.02	0.03 ± 0.01
2	10	37.26 ± 0.64	0.06 ± 0.01	0.02 ± 0.00
3	10	39.88 ± 0.55	0.12 ± 0.02	0.02 ± 0.00
4	10	34.81 ± 0.75	0.06 ± 0.01	0.02 ± 0.01

5	10	38.11 ± 1.04	0.04 ± 0.01	0.02 ± 0.00
6	10	39.19 ± 0.77	0.05 ± 0.01	0.02 ± 0.00
Average		37.85 ± 1.80	0.07 ± 0.03	0.02 ± 0.01
LOD (3SD)		5.41	0.09	0.02
LOQ (10SD)		18.04	0.29	0.08

Table S9. Number of injections, concentration (ng g^{-1}), LOD and LOQ of L-Arg, ADMA and SDMA obtained from the measurement of 3 replicates of low concentration samples. Uncertainty of the values correspond to the standard deviation of the concentration obtained for the replicates indicated.

Replicates	**Injections**	**Concentration (ng g^{-1})**		
		L-Arginine	**ADMA**	**SDMA**
1	3	1325.55 ± 4.98	30.53 ± 0.57	48.62 ± 1.34
2	3	1386.18 ± 3.83	30.98 ± 0.40	50.80 ± 0.71
3	3	1392.89 ± 1.54	30.69 ± 0.51	51.52 ± 0.92
Average		1368.21 ± 32.29	30.73 ± 0.48	50.31 ± 1.58
LOD (3SD)		96.86	1.43	4.75
LOQ (10SD)		322.87	4.76	15.83

Figure S7. 1D-HPLC-MS/MS total ion chromatogram of a 1:5 dilution of a human pooled plasma with PBS containing 1.37 µg g^{-1} of L-Arg, 0.03 µg g^{-1} of ADMA and 0.05 µg g^{-1} of SDMA and spiked with 3.56 µg of $^{15}N_4$-arginine, 0.19 µg of $^{2}D_7$-ADMA and 0.33 µg of $^{2}D_6$-SDMA.


x10 4
7
6
5
4
3
2
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SDMA
L-arginine
ADMA
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6
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Counts vs. Acquisition time