This is supplementary material to the pre-peer reviewed version of the following article: Mona M. Khamis, Darryl J. Adamko and Anas El-Aneed. (2017), Development of a validated LC- MS/MS method for the quantification of 19 endogenous asthma/COPD potential urinary biomarkers. Analytica Chimica Acta, 989: 45-58. which has been published in final form at doi: 10.1016/j.aca.2017.08.007. This article may be used for non-commercial purposes in accordance with Elsevier and conditions for self-archiving.

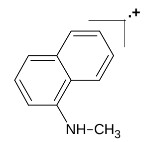
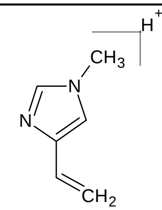
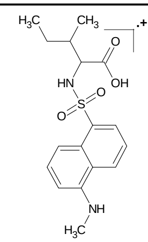
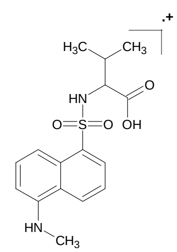
Supplemental Tables

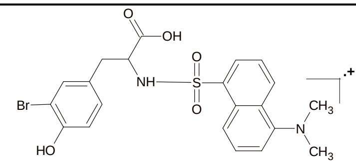
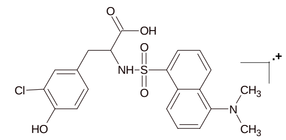
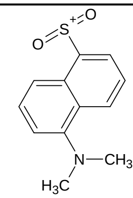
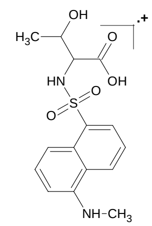
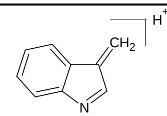
Development and validation of a novel LC- MS/MS method for the quantification of nineteen endogenous asthma/COPD urinary biomarkers

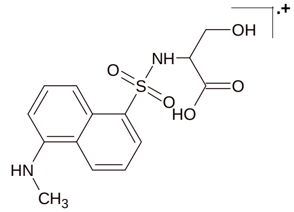
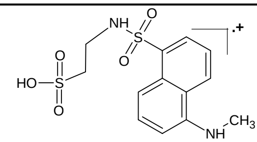
Table 1: Chromatographic and MS parameters of the 19 studied $^{12}C_2$- analytes

Chromatography: Autosampler 4 ºC, injection volume 5 µL, column: Kinetex C18 (100 mm×2.1 mm, 5 µm ID, 100 Å), at 22 ºC, mobile phase: (A) 0.1% formic acid in 5% ACN and (B) 0.1% formic acid in ACN, gradient system: t=0 min; 90% A, t=6 min; 70% A, t=19 min; 35% A, t=23 min; 1% A, t=24 min; 1% A, t=24.1 min; 90% A, t=34 min; 90% A, 0.25 mL/min.

Mass spectrometry: $^{13}C_2$-ISs monitored at m/z $[M+H]^+ \rightarrow$ m/z 172.10, Ion source temperature: 550 ºC, voltage: 5.5 kV, CUR: 30, CAD: 6, GS1, GS2: 50, dwell time: 20 msec, cycle time: 1.4504 sec. Data processing: Analyst® 1.6 software.

$^{12}C_2$- Analyte	Abbreviation	Q1 (Da)	Q3 (Da) Qualifier*	Suggested structure of qualifier fragment	Collision Energy (CE)	Declustering potential (DP)
Sarcosine-DNS	SAR	323.11	157.09		27	66
1-methylhistamine	1MH	359.15	109.08		41	75
Isoleucine-DNS	ISO	365.15	350.13		31	65
Valine-DNS	VAL	351.14	336.11		30	75

3-bromotyrosine-2DNS	3BrTyr	726.09	492.03		50	70
3-chlorotyrosine-2DNS	3ClTyr	682.14	448.09		50	70
Histidine-2DNS	HIS	622.18	234.06		47	85
Threonine-DNS	THRE	353.12	338.09		30	70
Tryptophan-DNS	TRP	438.15	130.07		39	60
Alanine-DNS	ALA	323.10	157.09	Same as SAR	29	73
Glutamine-DNS	GLU	380.13	234.06	Same as HIS	37	60
Tyrosine-2DNS	TYR	648.18	234.06	Same as HIS	50	90

Serine-DNS	SER	339.10	324.08		29	70
Taurine-DNS	TAU	359.07	344.05		35	70
Arginine-DNS	ARG	408.17	234.06	Same as GLU	45	70
Glycine-DNS	GLY	309.10	157.09	Same as SAR	29	75
Ethanolamine-DNS	EtNH2	295.11	157.09	Same as SAR	32	55
Asparagine-DNS	ASP	366.11	157.09	Same as SAR	35	70
Lysine-2DNS	LYS	613.21	234.06	Same as GLU	38	80

* Quantifier ion is fixed for all derivatized metabolites at m/z= 170.1

Table 2: Comparison of the slopes of the calibration curves generated in surrogate and real urine

Analyte	Slope in surrogate urine	Slope in real urine	t experimental*
SAR	3.72E-03	3.85E-03	0.986
1MH	2.01E-03	2.14E-03	2.08
ISO	1.74E-03	1.83E-03	1.35
VAL	1.83E-03	1.88E-03	1.55
3BrTyr	1.74E-03	1.79E-03	1.24
3ClTyr	2.32E-03	2.41E-03	1.09
HIS	2.15E-03	NA	NA
THRE	9.58E-04	9.11E-04	1.06
TRP	9.41E-04	9.25E-04	0.82
ALA	9.23E-04	9.50E-04	0.98
GLU	9.76E-04	9.18E-04	1.43
TYR	1.16E-03	1.13E-03	1.57
SER	4.46E-04	4.77E-04	1.61
ARG	4.87E-04	4.62E-04	1.99
GLY	5.18E-04	5.23E-04	0.31
EtNH2	4.38E-04	4.28E-04	1.28
TAU	5.05E-04	4.94E-04	0.57
ASP	4.54E-04	4.34E-04	1.48
LYS	2.97E-04	2.96E-04	0.18

*t critical= 2.179, 95% confidence level, $n_1=n_2=7$, $df=12$

Table 3: Evaluation of the matrix and carry over effects encountered in the developed method for the quantification of the 19 metabolites

Analyte	MF±RSD% (n=12)	IS normalized MF±RSD% (n=12)	Carry over (Blank peak area/LLOQ peak area ×100), n=6
SAR	1.01±5.0	0.97±7.6	0.4
1MH	0.82±10.6	0.95±5.2	3.1
ISO	0.96±5.53	0.96±7.7	7.4
VAL	1.02±5.23	0.96±5.6	10.6
3BrTyr	1.22±9.7	1.07±8.3	12.7
3ClTyr	1.15±9.5	0.97±7.4	10.6
HIS	1.17±5.1	0.97±5.1	13.5
THRE	1.01±4.9	0.94±6.4	11.2
TRP	1.08±11.8	1.00±7.7	7.8
ALA	1.02±5.9	0.97±6.9	6.2
GLU	1.00±4.0	0.95±6.92	7.9
TYR	1.10±4.1	0.96±7.4	16.3
SER	1.04±5.5	0.99±5.6	18.8
ARG	1.11±11.6	0.94±7.0	7.3
GLY	1.01±5.1	0.98±6.1	13.3
EtNH2	97.1±3.6	0.96±5.3	7.0
TAU	1.05±5.2	1.00±5.2	8.9
ASP	1.22±9.5	0.98±6.3	7.3
LYS	1.29±7.1	0.95±6.9	8.4

Table 4: Regression parameters for the determination of the 19 studied $^{12}C_2$- analytes using the developed method

Analyte	Concentration in working stock mixture (μg/mL)	Concentration range (ng/mL)	Regression equation	Coefficient of determination (R^2)	Concentration of IS (ng/mL)
SAR	12.5	1.875-375	y = 0.00379 x + 0.000623	0.9964	250
1MH	15.8	2.37-474	y = 0.00318 x + 0.003330	0.9964	315
ISO	25	3.75-750	y = 0.00191 x + 0.000841	0.9966	500
VAL	25	3.75-750	y = 0.00181 x + 0.001260	0.9962	500
3BrTyr	25	3.75-750	y = 0.00181 x + 0.000717	0.9974	500
3ClTyr	25	3.75-750	y = 0.00224 x + 0.000298	0.9972	500
HIS	25	3.75-750	y = 0.00199 x + 0.000032	0.9956	500
THRE	50	7.5-1500	y = 0.00893 x + 0.001110	0.9966	1000
TRP	50	7.5-1500	y = 0.00085 x + 0.000532	0.9972	1000
ALA	50	7.5-1500	y = 0.00091 x + 0.001040	0.9970	1000
GLU	50	7.5-1500	y = 0.00089 x + 0.001430	0.9974	1000
TYR	50	7.5-1500	y = 0.00106 x + 0.000901	0.9960	1000
SER	100	15-3000	y = 0.00042 x + 0.001760	0.9948	2000
ARG	100	15-3000	y = 0.00045 x + 0.000554	0.9958	2000
GLY	100	15-3000	y = 0.00049 x + 0.002450	0.9968	2000
EtNH2	100	15-3000	y = 0.00043 x + 0.003430	0.9978	2000
TAU	100	15-3000	y = 0.00046 x + 0.001510	0.9954	2000
ASP	100	15-3000	y = 0.00040 x + 0.001820	0.9956	2000
LYS	160	24-4800	y = 0.00038 x + 0.000504	0.9976	3200

Table 5: Evaluation of homoscedasticity of unweighted/weighted calibration curves

Analyte	*F*-statistical for homogeneity of variance*	Σ%RE		
		Unweighted	1/x	$1/x^2$
SAR	27266.5	349.8	48.2	33.8
1MH	83505.3	563.4	53.1	37.1
ISO	89424.8	273.2	48.5	35.1
VAL	110398.80	81.7	53.3	47.1
3BrTyr	44960.8	274.7	69.7	54.5
3ClTyr	35614.9	95.0	56.5	56.1
HIS	28172.4	649.9	81.1	48.5
THRE	60664.7	245.4	34.7	30.7
TRP	107373.5	72.3	51.7	47.4
ALA	168588.3	273.1	46.5	34.2
GLU	146190.5	138	43	38
TYR	73242.6	391.3	48.1	18.5
SER	89660.4	239.5	36.6	27.8
ARG	101065.7	527.5	68.4	59.3
GLY	184188.5	377.5	48.3	27.5
EtNH2	107391.5	326.2	39.4	32.2
TAU	113124.1	448.8	46.1	32.4
ASP	18317.3	313.8	34.4	33.7
LYS	1271572.0	68.9	25.1	24.5

*F critical; 5.05 at 95% confidence interval, df=5,5

Table 6: Intra-day precision and accuracy of the developed method for the quantification of the 19 $^{12}C_2$- analytes

Analyte	Day	Quality control level															
		LLOQ				**LQC**				**MQC**				**HQC**			
		Nominal (ng/mL)	Found (ng/mL)±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL)±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)
	1		1.78±0.10	92.6	5.69		5.17±0.08	103.2	1.56		158.4±2.19	101.8	1.38		297±9.22	95.0	3.10
SAR	2	**1.88**	1.92±0.23	85.0	11.84	**5**	5.19±0.24	103.7	4.68	**156**	169.2±7.73	108.4	4.57	**313**	316±29.52	101.0	9.34
	3		1.86±0.08	98.8	4.21		5.29±0.21	105.7	4.02		161.60±7.77	103.6	4.81		323±3.42	103.2	1.06
	1		2.08±0.19	87.8	9.28		6.13±0.25	96.3	4.14		199.2±4.66	101.3	4.60		386±16.01	97.8	4.17
1MH	2	**2.37**	2.45±0.17	103.5	7.10	**6.32**	6.66±0.35	105.5	5.32	**197**	214.6±3.85	109.2	1.79	**395**	375±38.15	95.0	10.16
	3		2.42±0.09	102.1	3.57		6.71±0.52	106.2	3.62		203.4±7.37	103.2	3.62		399±20.76	100.9	5.26
	1		3.68±0.09	98.2	2.55		10.33±0.62	103.3	0.60		317.6±11.00	101.6	3.47		609±17.22	97.4	2.83
ISO	2	**3.75**	3.65±0.27	97.2	7.33	**10**	10.92±0.64	109.2	5.84	**313**	343.8±8.75	109.6	2.55	**625**	598±48.17	109.6	2.55
	3		3.78±0.09	100.8	2.37		10.60±0.44	106.0	4.14		330.8±9.33	105.8	2.82		609.4±28.88	97.5	4.74
	1		3.46±0.16	92.3	4.53		10.51±0.72	105.1	6.81		324.6±4.62	103.8	1.42		608±13.33	97.2	2.19
VAL	2	**3.75**	4.07±0.24	108.3	5.92	**10**	10.86±0.24	108.6	2.22	**313**	337.4±11.61	107.8	3.44	**625**	623±53.44	99.8	8.57
	3		4.30±0.16	114.8	3.68		11.00±0.46	110.0	4.17		324.0±8.00	103.4	2.45		621±18.22	99.4	2.93
	1		3.73±0.60	99.4	16.14		9.94±0.92	99.4	9.25		315.0±10.50	100.7	3.34		625±16.04	100.2	2.56
3BrTyr	2	**3.75**	3.57±0.43	96.4	12.14	**10**	10.64±0.87	106.4	8.22	**313**	324.0±16.64	102.9	5.13	**625**	592±7.29	94.7	9.67
	3		4.01±0.21	107.0	5.35		9.79±0.89	97.9	9.11		323.0±22.53	102.3	6.98		624±21.89	99.86	3.51
	1		3.37±0.10	88.2	3.07		9.72±0.74	97.22	7.59		314.0±3.39	100.3	1.08		613±10.42	98.2	1.70
3ClTyr	2	**3.75**	3.67±0.47	97.8	12.94	**10**	10.4±0.68	104.0	6.56	**313**	326.0±8.92	104.0	2.74	**625**	635±63.17	101.6	9.95
	3		4.00±0.22	106.6	11.32		10.26±0.60	102.6	6.84		324.0±14.44	103.6	4.46		650±33.47	104.0	5.15
	1		3.61±0.16	96.3	4.33		9.95±0.22	99.5	2.19		313.2±3.56	99.9	1.14		623±22.63	99.7	3.63
HIS	2	**3.75**	3.68±0.30	98.1	8.14	**10**	9.85±0.57	98.5	5.79	**313**	335.2±14.60	107.2	4.36	**625**	638±25.24	102.1	3.95
	3		3.53±0.45	94.2	12.83		9.50±0.83	95.0	8.70		318.8±3.90	101.8	1.22		636±19.9	101.7	3.13
	1		7.15±0.23	95.3	3.26		21.02±0.50	105.2	2.36		626.8±12.24	100.3	1.95		1192±42.66	95.4	3.58
THRE	2	**7.5**	7.59±0.78	101.1	10.30	**20**	21.12±1.00	105.5	4.73	**625**	700.6±14.26	112.2	2.04	**1250**	1220±149.83	97.6	12.28
	3		8.16±0.35	108.8	4.30		21.36±0.65	106.8	3.04		656±48.25	104.8	7.36		1286±32.09	102.9	2.50
	1		7.21±0.12	96.1	1.63		20.18±1.07	100.9	5.28		643.8±16.21	103.0	2.52		1218±34.21	97.44	2.81
TRP	2	**7.5**	7.75±0.51	103.2	6.63	**20**	21.68±1.49	108.3	6.88	**625**	657.2±20.95	105.3	3.19	**1250**	1150±122.88	92.0	10.69
	3		7.42±0.43	98.9	5.85		20.18±0.80	100.9	3.99		648.8±20.34	103.6	3.13		1258±48.17	100.6	3.83

ALA	1		7.44±0.20	99.3	2.71		20.58±1.66	103.0	8.06		635.8±8.35	101.8	1.31		1178±22.80	94.2	1.94
	2	**7.5**	8.31±0.33	110.8	4.03	**20**	21.26±1.26	106.2	5.93	**625**	625.8±23.44	104.5	3.59	**1250**	1175±108.90	94.0	9.27
	3		7.78±0.15	103.6	1.93		20.28±0.75	101.5	3.68		634.6±27.04	101.6	4.26		1232±37.01	98.6	3.00
GLU	1		6.96±0.22	92.7	3.09		19.82±0.51	99.2	2.56		625.2±10.94	100.0	1.75		1208±29.50	96.7	2.44
	2	**7.5**	6.92±0.36	92.3	5.25	**20**	21.4±1.03	107.0	4.80	**625**	677.8±26.24	108.4	3.87	**1250**	1208±87.01	96.6	7.20
	3		7.51±0.54	100.0	7.21		20.1±1.82	100.4	9.03		660.8±44.97	105.6	6.81		1238±75.96	99.0	6.14
TYR	1		6.76±0.24	90.1	3.61		20.08±1.12	100.3	5.57		651.2±3.11	104.4	0.48		1258±41.47	100.6	3.30
	2	**7.5**	7.59±0.17	101.4	2.27	**20**	20.06±1.30	100.2	6.46	**625**	614.4±36.14	102.6	5.63	**1200**	1214±8.69	97.2	8.69
	3		7.71±0.33	102.7	4.21		19.68±0.83	98.4	4.24		638.6±27.61	104.3	4.32		1226±23.02	98.1	1.88
SER	1		13.68±0.30	91.1	2.22		41.84±0.45	104.6	1.08		1250±41.83	99.9	3.35		2430±108.00	97.1	4.46
	2	**15**	14.86±1.01	99.0	6.77	**40**	42.48±2.25	106.2	5.30	**1250**	1368±47.64	109.4	3.48	**2500**	2416±204.52	96.7	8.47
	3		14.76±0.69	98.4	4.66		41.74±1.93	104.3	4.64		1336±72.32	106.8	5.41		2618±35.64	104.6	1.36
ARG	1		14.20±0.57	94.6	4.01		41.04±2.42	102.7	5.89		1252±46.04	100.3	3.68		2488±59.80	99.6	2.40
	2	**15**	14.54±0.88	97.0	6.03	**40**	41.76±2.21	104.5	5.30	**1250**	1304±42.19	104.2	3.24	**2500**	2414±300.13	96.5	12.43
	3		15.20±063	101.0	4.13		42.54±2.69	106.4	6.30		1254±45.06	100.5	3.59		2616±198.70	104.6	7.60
GLY	1		13.50±0.37	90.0	2.72		40.30±1.71	100.8	4.24		1252±17.89	100.4	1.43		2388±61.40	95.5	2.57
	2	**15**	16.06±0.88	106.8	5.47	**40**	42.22±2.30	105.5	5.46	**1250**	1366±55.50	109.2	4.06	**2500**	2488±326.00	99.4	13.10
	3		14.30±0.52	95.4	3.67		40.52±1.97	101.3	4.87		1306±66.56	104.8	5.10		2462±36.33	98.4	1.48
EtNH2	1		14.06±0.82	93.8	5.83		40.96±2.20	102.5	5.36		1256±21.91	100.4	1.74		2404±76.35	96.1	3.18
	2	**15**	14.66±0.60	97.6	4.11	**40**	41.48±2.59	103.7	6.25	**1250**	1390±62.45	111.0	4.49	**2500**	2392±199.80	95.7	8.35
	3		15.54±0.82	103.5	5.30		40.42±1.17	101.0	2.89		1300±57.88	104.0	4.45		2560±59.58	102.5	2.33
TAU	1		14.34±0.43	95.6	3.02		40.70±1.99	101.9	4.90		1268±42.07	101.4	3.32		2496±72.32	99.7	2.90
	2	**15**	15.98±1.08	106.6	6.7	**40**	44.12±1.41	110.2	3.20	**1250**	1338±39.62	107.0	2.96	**2500**	2456±181.88	98.2	7.41
	3		16.32±1.00	108.9	6.12		39.28±2.21	98.36	5.62		1314±45.01	105	3.43		2558±178.10	102.1	6.97
ASP	1		13.46±0.39	89.6	2.91		39.36±1.28	98.3	3.25		1244±18.17	99.6	1.46		2416±88.77	96.6	3.67
	2	**15**	14.90±2.17	99.4	14.57	**40**	42.02±1.66	105.2	3.96	**1250**	1342±60.99	107.4	4.54	**2500**	2458±234.35	98.3	9.53
	3		15.26±0.95	101.7	6.24		41.34±1.78	103.6	4.31		1334±39.12	106.8	2.93		2562±38.34	102.8	1.50
LYS	1		23.70±0.40	98.8	1.69		66.58±4.22	103.9	6.34		1986±27.02	99.3	1.36		3844±48.27	96.2	1.26
	2	**24**	23.66±0.52	98.6	2.19	**64**	69.08±2.27	108.0	3.28	**2000**	2070±29.15	103.6	1.41	**4000**	3854±425.24	96.4	11.03
	3		23.38±0.33	97.4	1.43		67.72±2.13	105.8	3.15		2030±77.14	101.5	3.80		3712±142.20	92.8	3.83

Table 7: Inter-day precision and accuracy of the developed method for the quantification of the 19 $^{12}C_2$- analytes

Analyte	Quality control level*															
	LLOQ				**LQC**				**MQC**				**HQC**			
	Nominal (ng/mL)	Found (ng/mL)±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL)±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)
SAR	**1.88**	1.87±0.15	99.3	8.0	**5**	5.19±0.18	103.7	3.5	**156**	162.17±7.6	104.0	4.7	**313**	309.08±25.25	98.8	8.17
1MH	**2.37**	2.32±0.23	97.78	9.8	**6.32**	6.51±0.45	102.9	7.0	**197**	205.73±8.44	104.4	4.1	**395**	386.8±26.68	97.9	6.90
ISO	**3.75**	3.73±0.17	99.3	4.6	**10**	10.62±0.59	106.2	5.5	**313**	330.7±14.3	105.6	4.3	**625**	605.5±31.9	96.9	5.3
VAL	**3.75**	3.9±0.41	105.2	10.3	**10**	10.79±0.52	107.9	4.8	**313**	328.67±10.2	105.0	3.1	**625**	614.5±31.9	98.8	5.2
3BrTyr	**3.75**	3.77±0.45	100.5	12.1	**10**	101.12±0.91	101.2	9.0	**313**	319.07±16.3	101.9	5.1	**625**	614.07±37.4	98.3	6.1
3ClTyr	**3.75**	3.69±0.39	98.1	10.6	**10**	10.13±0.70	101.3	6.8	**313**	321.3±10.7	102.66	3.3	**625**	632.7±41.8	101.2	6.6
HIS	**3.75**	3.61±0.31	96.21	8.6	**10**	9.77±0.58	97.66	6.0	**313**	322.4±12.7	103.0	4.0	**625**	632.3±22.0	101.2	3.5
THRE	**7.5**	7.63±0.64	101.8	8.4	**20**	21.17±0.71	105.83	3.3	**625**	661.0±41.9	105.8	6.3	**1250**	1232.7±94.3	98.6	7.7
TRP	**7.5**	7.46±0.43	99.4	5.8	**20**	20.68±1.30	103.4	6.3	**625**	649.9±18.7	104.0	2.9	**1250**	1208.7±82.3	96.7	7.1
ALA	**7.5**	7.85±0.43	104.6	5.5	**20**	20.71±1.26	103.5	6.1	**625**	641.1±21.4	102.6	3.3	**1250**	1199.3±70.8	99.9	5.9
GLU	**7.5**	7.13±0.46	95.0	6.4	**20**	20.44±1.35	102.2	6.6	**625**	653.4±38.1	104.5	5.8	**1250**	1218±65.4	97.4	5.4
TYR	**7.5**	7.35±0.50	98.03	6.8	**20**	19.94±1.04	99.7	5.2	**625**	643.7±25.0	103.0	3.9	**1200**	1232.7±64.7	98.6	5.3
SER	**15**	14.43±0.87	96.2	6.0	**40**	42.02±1.64	105.1	3.9	**1250**	1318±72.8	105.4	5.5	**2500**	2488±157.4	99.52	6.3
ARG	**15**	14.91±0.93	99.4	6.3	**40**	41.78±2.35	104.5	5.6	**1250**	1270±48.1	101.6	3.8	**2500**	2506±213.3	100.2	8.5
GLY	**15**	14.62±1.25	97.5	8.6	**40**	41.39±1.86	103.5	4.5	**1250**	1308±67.5	104.6	5.1	**2500**	2446±183.6	97.8	7.5
EtNH2	**15**	14.75±0.94	98.4	6.4	**40**	40.95±1.97	102.4	4.8	**1250**	1315±75.4	105.2	5.7	**2500**	2452±142.7	98.1	5.8
TAU	**15**	15.54±1.21	103.6	7.8	**40**	41.37±2.74	103.4	6.6	**1250**	1306.7±49.4	104.5	3.8	**2500**	2503±147.9	100.1	5.9
ASP	**15**	14.54±1.52	96.7	10.43	**40**	40.9±1.88	102.3	4.5	**1250**	1306.7±60.9	104.5	4.7	**2500**	2478±149.7	99.14	6.0
LYS	**24**	23.58±0.42	98.25	1.8	**64**	67.79±3.00	105.9	4.4	**2000**	2028.7±58.4	101.4	2.9	**4000**	3803±250.2	95.1	6.6

Table 8: Stability of the developed method for the quantification of the 19 $^{12}C_2$- analytes

Analyte	Stability	Quality Control							
		LQC				HQC			
		Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)
SAR	48 h 4°C	**5**	5.15±0.34	104.2	6.56	**313**	314.0±14.46	100.4	4.6
	3 FT cycles		4.83±0.29	96.6	6.01		305.0±24.68	97.5	8.09
	Bench RT 4h		5.40±0.18	108.0	3.41		320.2±13.12	102.2	4.10
	2 Weeks		5.67±0.12	113.4	2.13		307.5±13.28	98.2	4.32
	3 Months		4.13±0.33	82.6	8.07		347.2±4.38	110.9	1.26
	Stock stability		5.07±0.22	101.3	4.29		296.6±44.46	94.7	3.89
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			198±101.56	NA	51.3				
1MH	48 h 4°C	**6.32**	6.42±0.25	101.5	3.93	**395**	99.64±2.4	99.6	2.37
	3 FT cycles		6.10±0.65	96.4	10.52		409.8±23.45	103.6	5.72
	Bench RT 4h		6.65±0.19	105.2	2.89		406.8±14.45	102.8	3.55
	2 Weeks		6.87±0.39	108.7	5.73		389.3±9.78	98.5	2.51
	3 Months		4.93±1.73	77.95	2.22		361.4±3.79	91.5	4.14
	Stock stability		5.98±0.71	94.6	11.8		367.2±25.43	93.0	6.93
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			163.72±14.06	NA	8.59				
ISO	3 Months	**10**	10.54±0.45	105.4	4.28	**625**	607.4±16.56	97.2	2.73
	3 FT cycles		10.0±0.62	99.7	6.22		610.4±33.3	97.06	5.46
	Bench RT 4h		10.78±0.36	107.8	3.31		658.8±12.48	105.6	1.89
	2 Weeks		10.63±0.13	106.3	1.18		660.3±28.8	105.6	4.4
	3 Months		8.12±0.33	81.2	4.04		582.5±12.9	93.2	13.0
	Stock stability		9.93±0.27	99.28	2.75		592.6±31.32	94.82	5.3
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			709.6±48.63	NA	6.85				

VAL	48 h 4°C	**10**	11.28±0.30	112.8	2.69	**625**	614.8±17.94	98.36	2.92
	3 FT cycles		10.01±0.40	100.44	4.03		632.2±44.35	101.26	7.01
	Bench RT 4h		10.8±0.38	108.0	3.53		658.4±10.88	105.4	1.65
	2 Weeks		10.5±0.22	105.3	2.1		623.5±20.3	99.76	3.3
	3 Months		7.93±0.30	79.33	3.82		585.9±10.6	93.8	1.8
	Stock stability		9.78 ±0.32	97.74	3.31		580.4 ±27.52	92.86	4.74
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			2928±259	NA	8.85				
3BrTyr	48 h 4°C	**10**	9.54±0.53	95.44	5.57	**625**	597±16.48	95.5	2.76
	3 FT cycles		9.25±0.48	92.5	5.14		629.8±59.79	102.4	9.49
	Bench RT 4h		9.49±0.69	94.9	7.27		634.0±32.56	101.4	5.14
	2 Weeks		9.6±0.30	95.6	3.1		619.8±25.6	99.16	4.1
	3 Months		8.09±0.88	80.9	10.9		571.7±20.5	91.5	3.6
	Stock stability		8.60 ±0.87	85.96	10.15		585.6 ±24.7	93.7	4.2
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			<LLOQ	NA	NA				
3ClTyr	48 h 4°C	**10**	10.28±0.29	102.8	2.83	**625**	642.2±44.8	102.6	6.97
	3 FT cycles		9.63±0.42	96.3	4.24		652.2±70.93	104.3	10.88
	Bench RT 4h		10.45±0.56	104.5	5.37		684.4±36.83	109.4	5.38
	2 Weeks		10.1±0.67	100.6	6.7		639.5±32.7	102.3	5.1
	3 Months		8.17±0.52	81.7	6.43		556.9±40.05	89.1	7.19
	Stock stability		9.72 ±0.58	97.2	6.0		605.6 ±40.5	96.9	6.9
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			<LLOQ	NA	NA				
HIS	48 h 4°C	**10**	10.03±0.87	100.32	8.69	**625**	600.6±36.69	96.0	6.11
	3 FT cycles		9.55±0.47	95.48	4.93		632.6±47.87	100.9	7.57
	Bench RT 4h		10.29±0.31	102.9	3.00		671.6±32.17	107.46	4.79
	2 Weeks		10.6±0.45	105.8	4.3		628.5±11.8	100.6	1.9
	3 Months		7.79±0.38	77.9	4.93		533.12±16.7	85.3	3.13
	Stock stability		8.72 ±0.95	87.16	10.9		565.4 ±20.9	90.46	3.70

	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			20280±1961	NA	9.67				
THRE	48 h 4°C	**20**	21.88±4.6	109.2	4.22	**1250**	1244.0±3.91	99.52	0.31
	3 FT cycles		20.00±1.41	99.64	7.05		1208±109.2	96.76	9.03
	Bench RT 4h		21.64±0.81	108.2	3.75		1332.0±77.91	106.4	5.85
	2 Weeks		20.9±0.70	104.5	3.7		1300±46.9	104	3.61
	3 Months		16.82±0.92	84.1	5.48		1214.0±35.3	97.13	2.91
	Stock stability		18.92 ±0.61	94.6	3.25		1178 ±13.04	98.17	1.11
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			6856±860	NA	12.55				
TRP	48 h 4°C	**20**	21.26±1.18	106.4	5.55	**1250**	1250.0±57.18	100.0	4.58
	3 FT cycles		21.62±1.22	108.1	5.62		1258.0±146.18	100.7	11.62
	Bench RT 4h		21.18±0.61	105.6	2.88		1312.0±60.58	104.9	4.62
	2 Weeks		20.9±0.70	104.5	3.36		1300±46.9	104	3.61
	3 Months		17.2±0.83	86.03	2.18		1134.7±15.4	90.8	1.35
	Stock stability		19.24 ±0.61	96.2	3.20		1228 ±37.68	102.3	3.07
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			6256±695.2	NA	11.11				
ALA	48 h 4°C	**20**	21.20±0.86	106.2	4.02	**1250**	1230.0±35.36	98.4	2.87
	3 FT cycles		19.26±0.82	96.3	4.28		1304.0±139.75	104.28	10.72
	Bench RT 4h		21.68±0.54	108.4	2.47		1292.0±27.75	103.0	2.15
	2 Weeks		21.98±0.55	109.9	2.50		1280±8.2	102.4	0.63
	3 Months		16.6±0.44	83.0	2.65		1161.9±28.1	93.0	2.4
	Stock stability		19.12 ±1.40	95.6	7.31		1142 ±37.01	95.17	3.24
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			13368±1240	NA	9.27				
GLU	48 h 4°C	**20**	20.7±0.83	103.6	4.00	**1250**	1298.0±54.95	108.2	4.23
	3 FT cycles		19.90±1.64	99.44	8.22		1176.0±61.89	93.9	5.26
	Bench RT 4h		21.8±0.84	109.0	3.83		1322.0±51.9	105.8	3.92
	2 Weeks		21.0±0.43	104.8	2.1		1307.5±67.0	104.6	5.12

	3 Months		17.86±0.74	89.3	4.13		1266.3±55.28	101.3	4.4
	Stock stability		18.3 ±0.61	94.6	3.24		1178 ±53.20	97.83	4.53
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			28880±3898	NA	13.50				
TYR	48 h 4°C	20	20.96±0.44	104.8	2.10	1250	1234.0±35.07	98.7	2.84
	3 FT cycles		19.48±0.91	97.2	4.70		1220.0±106.77	97.68	8.75
	Bench RT 4h		21.06±0.86	105.2	4.08		1256.0±36.5	100.6	2.90
	2 Weeks		20.5±0.60	102.3	2.9		1257.5±61.3	100.6	4.9
	3 Months		15.11±0.32	75.55	2.09		1068.8±51.09	85.4	4.78
	Stock stability		18.22 ±0.97	91.1	5.30		1176 ±67.68	98.0	5.72
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			7576±676.4	NA	8.93				
SER	48 h 4°C	40	44.16±0.94	110.4	2.13	2500	2570±91.92	102.8	3.58
	3 FT cycles		40.5±1.80	101.1	4.44		2448±245.7	97.84	10.00
	Bench RT 4h		44.80±2.32	112.0	5.18		2502±80.44	100.3	3.21
	2 Weeks		45.78±0.46	114.4	0.99		2557±77.19	102.3	3.0
	3 Months		34.78±0.76	87.0	2.18		2344.4±37.93	93.8	1.6
	Stock stability		37.48 ±2.16	93.7	5.75		2350 ±77.46	94.0	3.30
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			17920±2036	NA	11.36				
ARG	48 h 4°C	40	42.48±1.23	106.0	2.89	2500	2472±69.79	98.9	2.82
	3 FT cycles		38.24±2.60	95.6	6.80		2516±300.45	100.5	11.94
	Bench RT 4h		43.0±1.34	107.4	3.12		2710.0±36.05	108.4	1.33
	2 Weeks		37.95±2.0	94.9	5.2		2490±191.3	99.6	7.7
	3 Months		31.8±20.47	79.6	1.47		2408.1±71.5	90.8	3.0
	Stock stability		37.88 ±5.64	94.7	14.88		2440 ±203.8	97.6	8.35
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			2092±334.2	NA	15.97				
GLY	48 h 4°C	40	43.98±1.62	109.8	3.69	2500	2448±77.27	97.9	3.16
	3 FT cycles		39.6±1.78	99.0	4.49		2414.0±123	96.54	5.10

	Bench RT 4h		44.7±1.21	111.8	2.70		2572.0±101.1	102.9	3.93
	2 Weeks		44.0±2.02	110	4.6		2525±67.5	101	2.7
	3 Months		33.7±1.11	84.3	3.29		2335.6±97.0	94.2	4.11
	Stock stability		39.2 ±1.15	98.0	2.93		2322 ±66.11	92.9	2.85
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			39120±3264	NA	8.34				
EtNH2	48 h 4°C	40	42.54±1.77	106.6	4.16	2500	2522±75.3	100.8	2.99
	3 FT cycles		37.74±2.07	94.4	5.48		2500±171.76	100.0	6.87
	Bench RT 4h		42.72±1.87	106.6	4.38		2632.0±50.7	105.4	1.93
	2 Weeks		42.8±2.6	107	6.02		2545±127.7	101.8	5.02
	3 Months		33.75±1.9	84.4	5.54		2363.1±76.0	94.5	3.2
	Stock stability		37.28 ±1.9	93.5	5.06		2360 ±67.45	94.4	2.86
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			28320±3.167	NA	11.19				
TAU	48 h 4°C	40	43.14±4.58	106.7	4.57	2500	2564±5.93	102.5	0.23
	3 FT cycles		39.7±2.55	99.2	6.43		2426±126.7	97.0	5.22
	Bench RT 4h		42.0±1.82	108.8	4.34		2586.0±116.7	103.4	4.51
	2 Weeks		41.4±1.53	103.5	3.7		2605±52.6	104.2	2.0
	3 Months		34.5±1.3	86.3	3.7		2411.9±36.9	96.5	1.52
	Stock stability		36.76 ±2.60	92.0	7.06		2426 ±143.11	97.04	5.9
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			4588±479	NA	10.45				
ASP	48 h 4°C	40	43.06±1.75	107.8	4.07	2500	2516.0±58.99	100.7	2.34
	3 FT cycles		40.50±1.75	101.2	4.32		2268.0±148.22	90.8	6.54
	Bench RT 4h		41.6±1.88	104.12	4.51		2582.0±146.5	103.4	5.67
	2 Weeks		43.9±1.5	109.7	3.5		2395±41.2	95.8	1.72
	3 Months		34.77±1.34	86.9	3.8		2480.0±86.48	99.2	3.48
	Stock stability		36.74 ±2.0	91.9	5.4		2230 ±73.5	89.2	3.30
	4 Months		Metabolome stability (ng/ mL urine, n=4)						
			6308±939	NA	14.89				
	48 h 4°C	64	70.98±1.53	111.0	2.16	4000	3684.0±97.62	92.1	2.65

	3 FT cycles	58.28±2.05	91.08	3.51	3972.0±178.52	99.38	4.49
	Bench RT 4h	67.88±3.14	106.0	5.01	4276.0±133.0	106.8	3.11
	2 Weeks	68.4±1.87	106.8	2.7	3922.5±76.32	98.06	1.95
LYS	3 Months	53.68±1.60	83.09	3.0	3486.0±119.0	87.2	3.42
	Stock stability	69.64 ±2.3	108.8	3.3	3564 ±153.3	89.1	4.30
		Metabolome stability (ng/ mL urine, n=4)					
	4 Months	1852±85.04	NA	4.60			

Table 9: Dilution integrity19 $^{12}C_2$- analytes spiked in the blanks surrogate matrix

Analyte	Concentration in spiked sample (µg/mL)	Folds of dilution											
		10 Fold				20 Fold				40 Fold			
		Nominal (ng/mL)	Found (ng/mL)±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)	Nominal (ng/mL)	Found (ng/mL) ±SD	Accuracy (%)	CV (%)
SAR	**2.5**	**250**	257.6±6.35	103.0	2.46	**125**	127.0±8.86	101.6	7.0	**62.5**	65.44±3.06	104.6	4.67
1MH	**3.15**	**315**	314.2±12.48	99.74	3.97	**158**	162.4±9.37	102.9	5.77	**79**	82.94±3.63	105.0	4.37
ISO	**5**	**500**	478.9±16.68	95.72	3.49	**250**	253.4±22.83	101.2	9.0	**125**	135.0±5.8	108.2	4.31
VAL	**5**	**500**	498.2±8.90	99.16	1.79	**250**	256.6±17.67	102.7	6.89	**125**	134.8±4.27	107.8	3.16
3BrTyr	**5**	**500**	493.8±13.22	98.8	2.68	**250**	255.8±17.02	102.5	6.65	**125**	127.8±2.59	102.0	2.03
3ClTyr	**5**	**500**	505.8±15.64	101.2	3.09	**250**	261.8±21.55	104.5	8.22	**125**	125.0±6.08	99.9	4.87
HIS	**5**	**500**	487.0±10.0	97.38	2.05	**250**	248.0±8.0	99.24	3.22	**125**	119.8±7.25	95.92	6.06
THRE	**10**	**1000**	1018±33.0	101.82	3.24	**500**	516.4±52.64	103.2	10.19	**250**	270.2±9.33	108.4	3.46
TRP	**10**	**1000**	962.8±35.32	96.28	3.67	**500**	488.0±23.96	97.54	4.90	**250**	261.0±11.81	104.2	4.53
ALA	**10**	**1000**	974.6±46.02	97.46	4.72	**500**	492.8±41.14	98.72	8.34	**250**	269.6±15.37	107.6	5.70
GLU	**10**	**1000**	1053.4±56.81	105.3	5.34	**500**	500.2±61.68	100.0	12.33	**250**	273.2±9.78	109.2	3.58
TYR	**10**	**1000**	955.0±24.6	95.5	2.57	**500**	500.2±35.20	100.22	7.04	**250**	248.0±7.42	99.22	3.00
SER	**20**	**2000**	2116.0±35.07	105.8	1.66	**1000**	1031.6±99.0	103.16	9.70	**500**	552.4±21.17	110.6	3.83
ARG	**20**	**2000**	1938.0±69.07	96.88	3.56	**1000**	998.4±103.3	99.84	10.35	**500**	549.4±30.5	109.9	5.55
GLY	**20**	**2000**	1974.0±55.05	98.5	2.79	**1000**	989.6±78.55	98.96	7.93	**500**	544.6±5.59	108.6	1.03
EtNH2	**20**	**2000**	1032.8±66.70	103.3	6.46	**1000**	1998.0±44.38	99.84	2.22	**500**	543.6±24.06	108.8	4.43
TAU	**20**	**2000**	2038.0±72.1	101.84	3.53	**1000**	1026.4±85.4	102.6	8.32	**500**	528.2±18.86	105.4	3.57
ASP	**20**	**2000**	1940.0±66.7	97.1	3.44	**1000**	999.6±109.7	100.0	10.98	**500**	534.8±27.33	107.0	5.10
LYS	**32**	**3200**	2962.0±81.36	92.5	2.74	**1600**	1632.0±90.39	101.8	5.54	**800**	830.0±26.67	103.76	3.21