Supplementary material

^{15}N ethanolamine tagging procedure

3 μL ^{15}N-ethanolamine (50 μmol) was added to the sample, the pH adjusted to 7.0 with 1 M HCl and DMT-MM (21 mg) was added to initiate the reaction[1,2]. The mixture was continuously stirred at room temperature for 4 hrs to complete the reaction. The pH was then adjusted to 5.0 by adding 1 M HCl or NaOH and the solutions were diluted to 600 μL by adding water or D_2O prior to detection by ^{1}H-^{15}N 2D NMR.[3,4]

^{13}C –formic acid tagging procedure

2 μL of ^{13}C-formic acid (0.05 mmol) and 5 mg of N-hydroxysuccinimide (0.04 mmol) were dissolved in 100 μL tetrahydrofuran. Nine mg of N, N-dicyclohexylcarbodiimide (0.04 mmol) in 50 μL tetrahydrofuran was added to the mixture and stirred at room temperature.[5] After 15 min, the reaction mixture was centrifuged to remove insoluble urea; the supernatant containing ^{13}C-N-formyloxysuccinimide was then added to the mixture of synthetic analogues of the metabolites along with 50 μL of 2 M $NaHCO_3$ (0.1 mmol) aqueous solution. The reaction was stirred at room temperature for 4 h and dried under vacuum. The residue was redispersed in 500 μL D_2O, the pH was adjusted to 7.0 by adding 1M HCl and then transferred to a standard 5mm NMR tube for analysis using ^{1}H-^{13}C 2D NMR.[6]

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5. Hecht, S. M.; Werner, D. *J. Chem. Soc. Perkin Trans. 1*, **1973**, 1903-1906.
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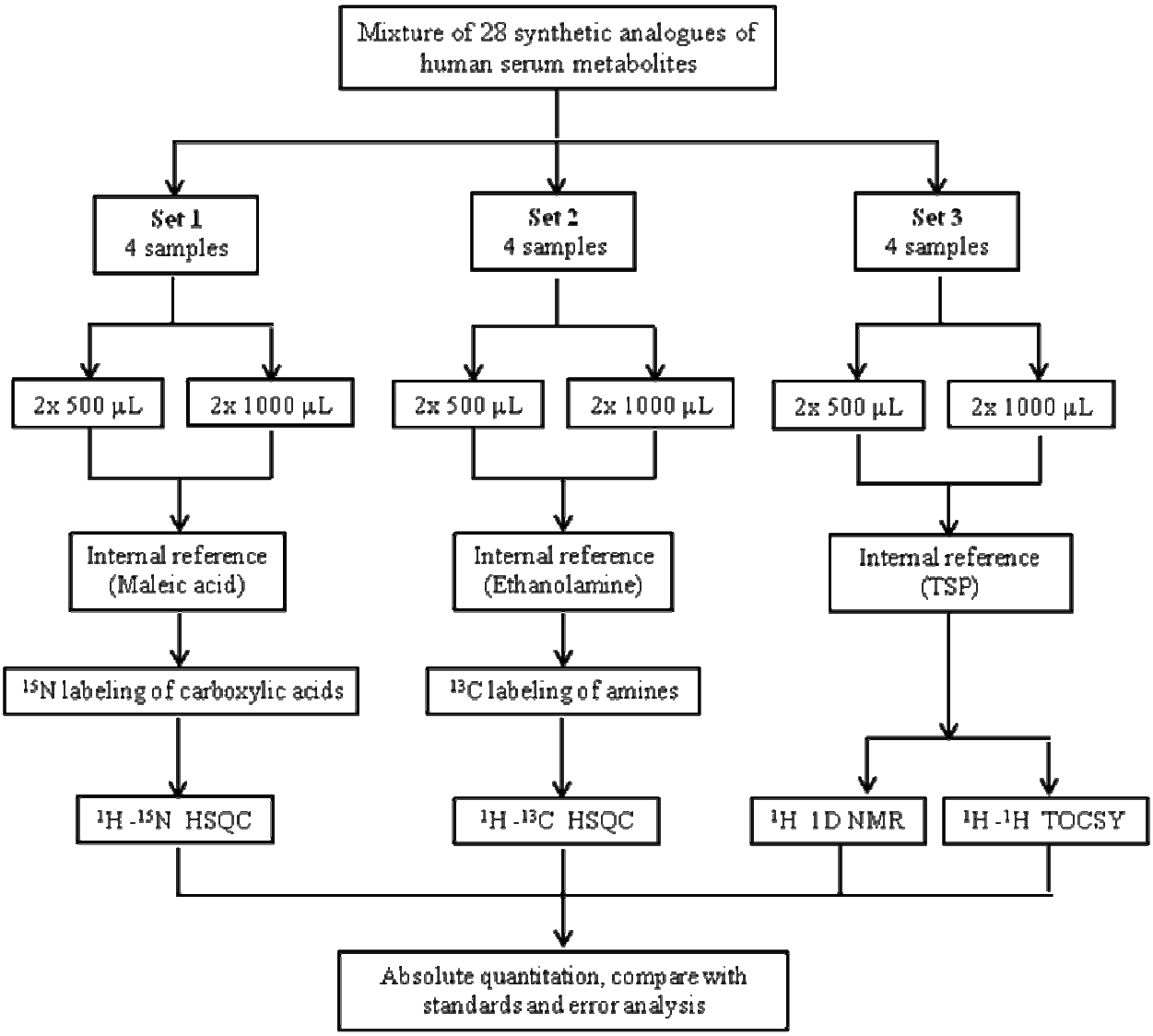
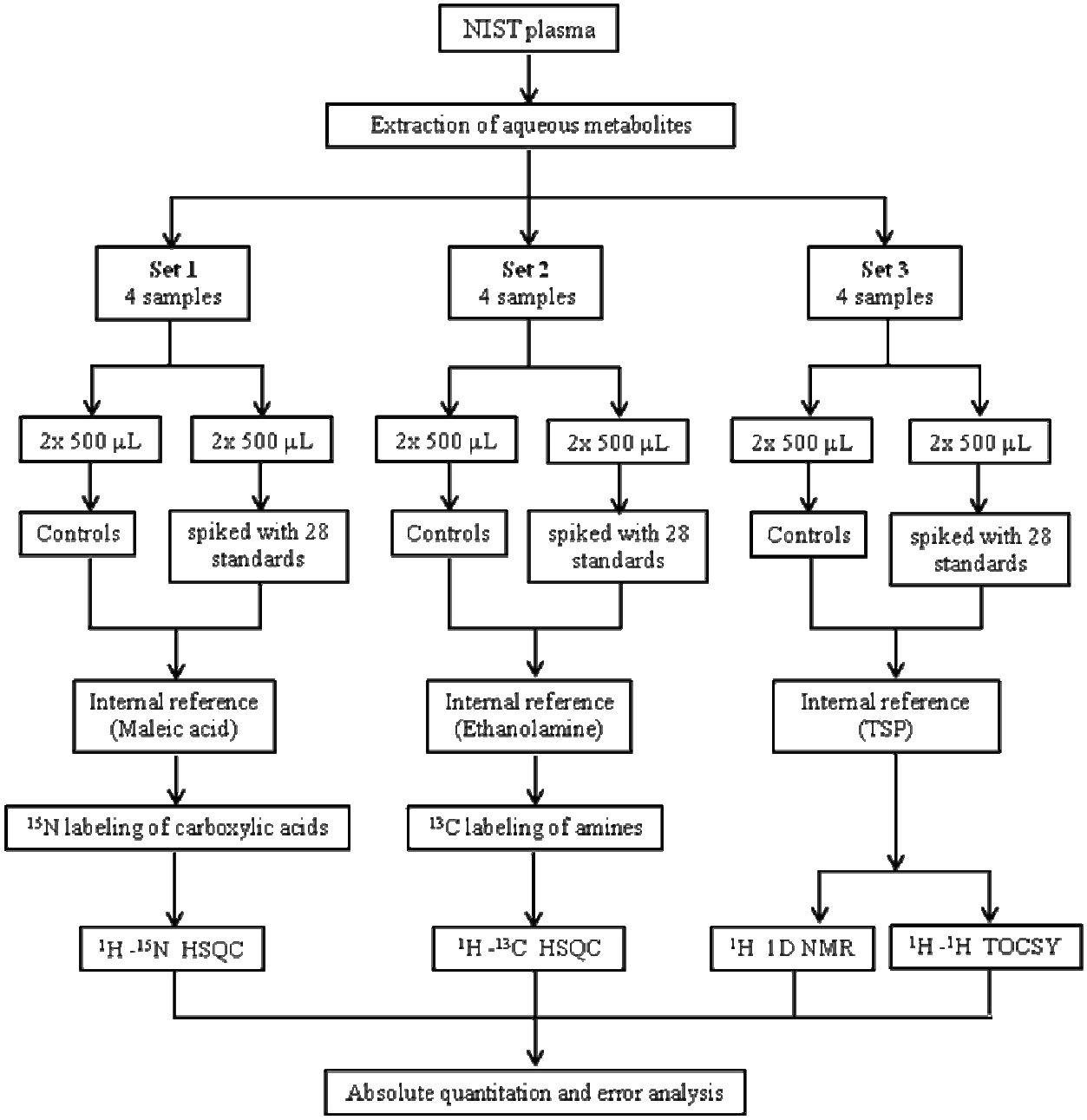


Flow Diagram 1: Flow chart depicting the steps followed in the analysis of the standard metabolite mixture using isotope enhanced NMR methods.



Flow Diagram 2: Flow chart depicting the steps followed in the analysis of metabolites in the NIST plasma sample using isotope enhanced NMR methods.

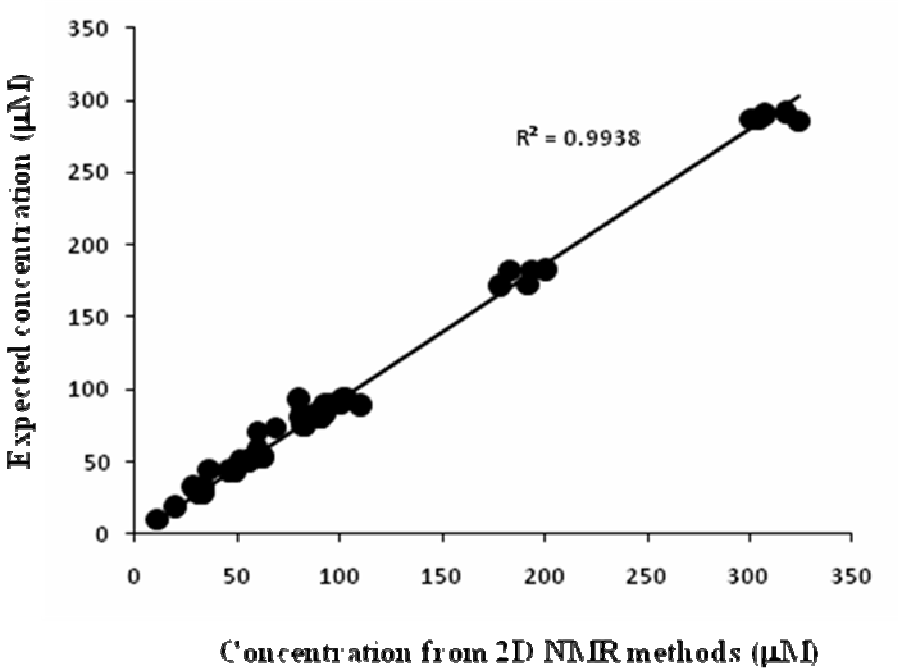


Figure S1: Correlation of the concentration of the metabolites determined by a combination 2D experiments with or without ^{15}N or ^{13}C tagging with the expected values.

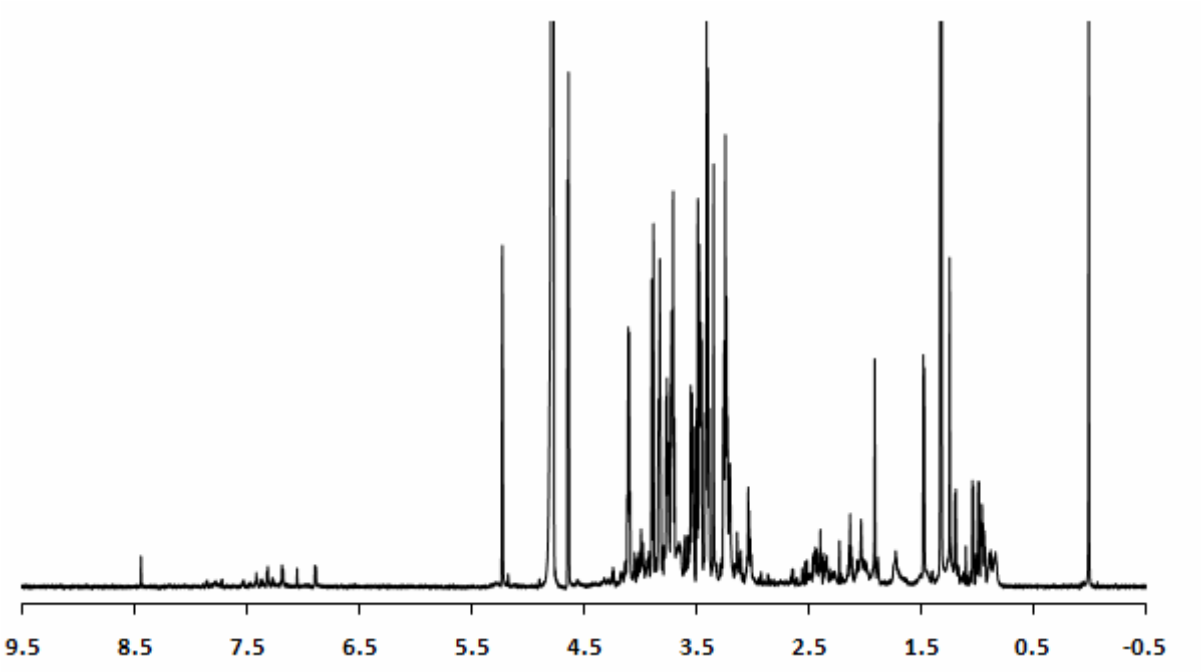


Figure S2: 1D ^{1}H NMR spectrum of aqueous metabolites of NIST plasma obtained on a Bruker 500 MHz NMR spectrometer.

Table S1: Concentration of synthetic analogues of human plasma metabolites before and after calibration using ^{1}H NMR.

Serial Number	Compound	Concentration from the weight (mM)*	Concentration after calibration by 1H NMR (mM)*
1	3-Hydroxybutyrate	20.02	20.69 ± 0.32
2	Acetate	19.62	19.33 ± 0.30
3	L-Alanine	19.67	20.07 ± 0.31
4	L-Arginine	20.00	23.62 ± 0.37
5	Citrate	20.09	19.67 ± 0.31
6	Creatinine	20.15	16.18 ± 0.30
7	Formate	20.38	17.02 ± 0.26
8	L-Glutamic acid	20.18	22.39 ± 0.12
9	L-Glutamine	20.15	20.33 ± 0.19
10	L-Glycine	20.58	18.09 ± 0.28
11	L-Histidine	20.10	21.83 ± 0.17
12	L-Isoleucine	20.24	21.07 ± 0.09
13	Lactate	20.21	20.12 ± 0.31
14	L-Leucine	20.37	21.16 ± 0.33
15	L-Lysine	20.11	15.98 ± 0.49
16	L-Methionine	19.95	19.96 ± 0.03
17	L-Phenylalanine	20.00	21.29 ± 0.33
18	L-Proline	20.16	19.57 ± 0.31
19	L-Threonine	19.98	18.95 ± 0.41
20	L-Tryptophan	20.00	20.36 ± 0.32
21	L-Tyrosine	19.87	21.04 ± 0.08
22	L-Valine	20.07	19.10 ± 0.15
23	Succinate	20.03	20.77 ± 0.33
24	Betaine	20.15	18.69 ± 0.30
25	4-hydroxy-proline	20.00	22.39 ± 0.35
26	L-Serine	19.98	19.45 ± 0.14
27	L-Asparagine	20.01	23.01 ± 0.36
28	Taurine	19.94	19.17 ± 0.07
30	Ethanolamine	20.00	20.34 ± 0.95
31	Maleic acid	20.07	20.85 ± 0.31
32	DSS	5.00	4.70 ± 0.02

DSS: 4,4-dimethyl-4-silapentane-1-sulfonic acid; * The errors are standard deviations based on two measurements.

Table S2: Synthetic analogues of human plasma metabolites grouped for concentration calibration using ^{1}H NMR.

Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
3-Hydroxybutyrate	L-Arginine	L-Glutamine	Creatinine	Succinate
Acetate	L-Histidine	L-Isoleucine	L-Glutamic acid	L-Serine
L-Alanine	Lactate	L-Threonine	L-Lysine	Taurine
Citrate	L-Phenylalanine	L-Tryptophan	L-Methionine	
Formate	L-Tyrosine	Ethanolamine	L-Proline	
L-Glycine	L-Valine			
L-Leucine	4-Hydroxy-proline			
Betaine	maleic acid			
L-Asparagine				