Supplementary Material

to

Cross-platform comparison of methods for quantitative metabolomics of primary metabolism

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Table S-1: Sorting of Metabolites into Categories. The CHEBI-IDs (http://www.ebi.ac.uk/chebi/init.do) of the uncharged molecule are given as unique identifiers.

Compounds	CHEBI-ID	Compound group	Number of compounds
phenylpyruvate	CHEBI:30851	amino acid and precursors: aromatic	4
phenylalanine	CHEBI:28044		
tyrosine	CHEBI:18186		
tryprophan	CHEBI:27897		
ornithine	CHEBI:18257	amino acid and precursors: basic	5
glutamine	CHEBI:28300		
arginine	CHEBI:29016		
citrulline	CHEBI:18211		
diaminopimelate	CHEBI:23673		
alanine	CHEBI:16449	amino acid and precursors: neutral	12
glyceraldehyde	CHEBI:5445		
serine	CHEBI:17822		
proline	CHEBI:26271		
valine	CHEBI:27266		
homoserine	CHEBI:30653		
cysteine	CHEBI:15356		
hydroxyproline	CHEBI:20392		
(iso)leucine	CHEBI:25017 and CHEBI:24898		
lysine	CHEBI:25094		
methionine	CHEBI:16811		
histidine	CHEBI:27570		
aspartate	CHEBI:22660	amino acids and precursors: acidic	2
glutamate	CHEBI:18237		
cytosine	CHEBI:16040	bases	5
uracil	CHEBI:17568		
thymine	CHEBI:17821		
adenine	CHEBI:16708		
guanine	CHEBI:16235		
coenzyme A	CHEBI:15346	coenzyme A esters	2

Compounds	CHEBI-ID	Compound group	Number of compounds
acetyl coenzyme A	CHEBI:15351		
cyclic AMP	CHEBI:17489	nucleotides	9
cyclic GMP	CHEBI:16356		
AMP	CHEBI:16027		
GMP	CHEBI:15996		
ADP	CHEBI:16761		
GDP	CHEBI:17552		
CTP	CHEBI:17677		
ATP	CHEBI:15422		
GTP	CHEBI:15996		
glyoxylate	CHEBI:16891	organic acids	17
pyruvate	CHEBI:32816		
oxalate	CHEBI:16995		
lactate	CHEBI:28358		
alpha & gamma aminobutyrate	CHEBI:35621 and CHEBI:16865		
fumarate	CHEBI:18012		
ketovaline	CHEBI:27266		
succinate	CHEBI:15741		
nicotinate	CHEBI:15940		
ketoisoleucine and ketoleucine	CHEBI:15614 and CHEBI:48430		
oxaloacetate	CHEBI:30744		
malate	CHEBI:6650		
alpha ketoglutarate	CHEBI:30915		
shikimate	CHEBI:16119		
(iso)citrate	CHEBI:30887 and CHEBI:30769		
glucuronate	CHEBI:24298		
pantothenate	CHEBI:7916		
NAD+	CHEBI:15846	redox cofactors	4
NADH	CHEBI:16908		
NADP+	CHEBI:18009		
NADPH	CHEBI:16474		

Compounds	CHEBI-ID	Compound group	Number of compounds
pentoses	CHEBI:25901	sugars	5
hexoses	CHEBI:18133		
disaccharides	CHEBI:36233		
mannitol	CHEBI:29864		
N-acetyl glucosamine	CHEBI:21638		
erythrose 4-P	CHEBI:48153	sugar phosphates	10
PEP	CHEBI:44897		
triose P	CHEBI:17138 and CHEBI:16108		
glycerol P	CHEBI:16890		
3-P glycerate	CHEBI:17050		
pentose P	CHEBI:17797 and CHEBI:26573		
hexose P	CHEBI:15965		
2,3-bisphospho glycerate	CHEBI:22902		
6-P gloconate	CHEBI:48928		
fructose bisphosphate	CHEBI:16905		

Table S-2: Number of quantifiable metabolites in pure standard mixture without added ^{13}C labeled biomass extract. Assignment of metabolites to classes, see table S-1.

Metabolite class	**Metabolites tested**	**GC**		**LC**		**CE**	
		TMS	**TBDMS**	**Ionpairing**	**HILIC**	**Bared silica**	**Polybrene**
neutral amino acids and precursors	12	10	10	6	9	7	6
acidic amino acids and precursors	2	2	2	1	1	1	2
basic amino acids and precursors	5	2	2	5	3	3	3
aromatic amino acids and precursors	4	4	2	3	3	3	2
organic acids	17	8	7	11	9	7	14
sugars	5	4	0	4	4	1	2
sugar phosphates	10	6	0	8	7	4	10
nucleotides	9	0	0	8	7	6	8
redox cofactors	4	0	0	4	4	2	4
coenzyme A esters	2	0	0	2	2	0	2
bases	5	4	0	5	5	5	3

Table S- 3: Selected chromatograms demonstrating the separability of isomers in the presence and absence of ^{13}C labeled biomass extract.

Hexoses are separable by GC-TMS in clean samples (solid line in (A)), but the presence of residual glucose in the ^{13}C labeled biomass extract strongly interferes with the separation and detection of hexoses (dashed line in A). The total peak are of hexoses is also greatly reduced in the sample with added ^{13}C labeled biomass extract as compared to the clean sample (0.58 10^7 counts and 2.21 10^7 counts, respectively) It is worth noticing that the separation of the chemically closely related disaccharides remains unaffected (solid and dashed lines in (B)).

The LC-HILIC method used in this study showed poor separation of sugars, even though separation of sugars by HILIC chromatography has been demonstrated before. As an example, the chromatogram of disaccharides is shown in (C). It seem likely that these sugars will be separable with an optimized gradient.

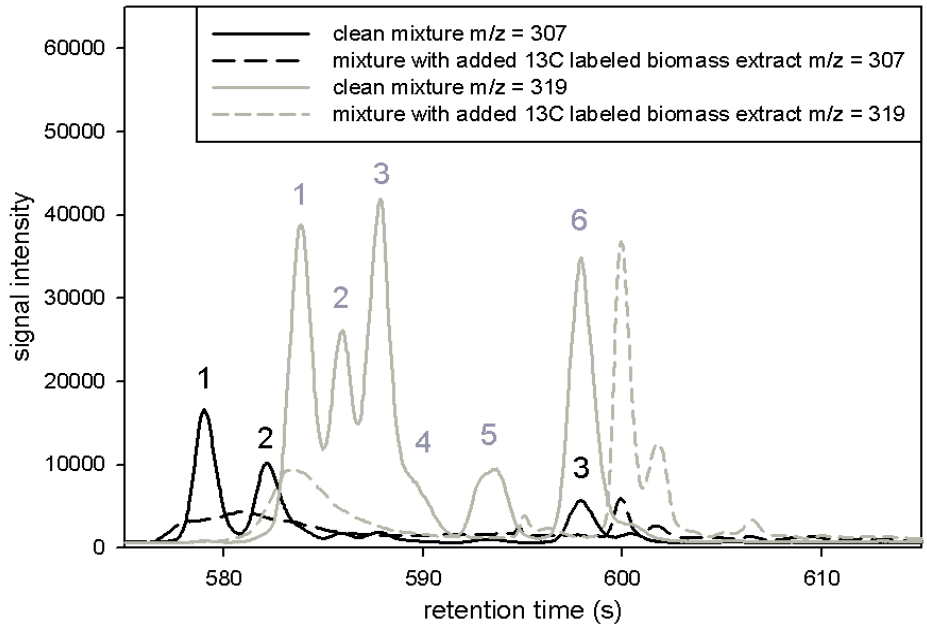
The separation of hexose phosphates by LC-ionpairing is corrupted by the presence of ^{13}C labeled biomass extract, which causes a broadening of the peaks (D). The total area of the peaks changes little (2.90 10^7 and 2.18 10^7 counts), indicating that ion suppression plays a minor role.

The influence of the derivatization agent can be seen comparing (E) and (F). While citrate and isocitrate are indistinguishable by GC-TMS, the respective peaks are nearly baseline-separated by GC-TBDMS.

A

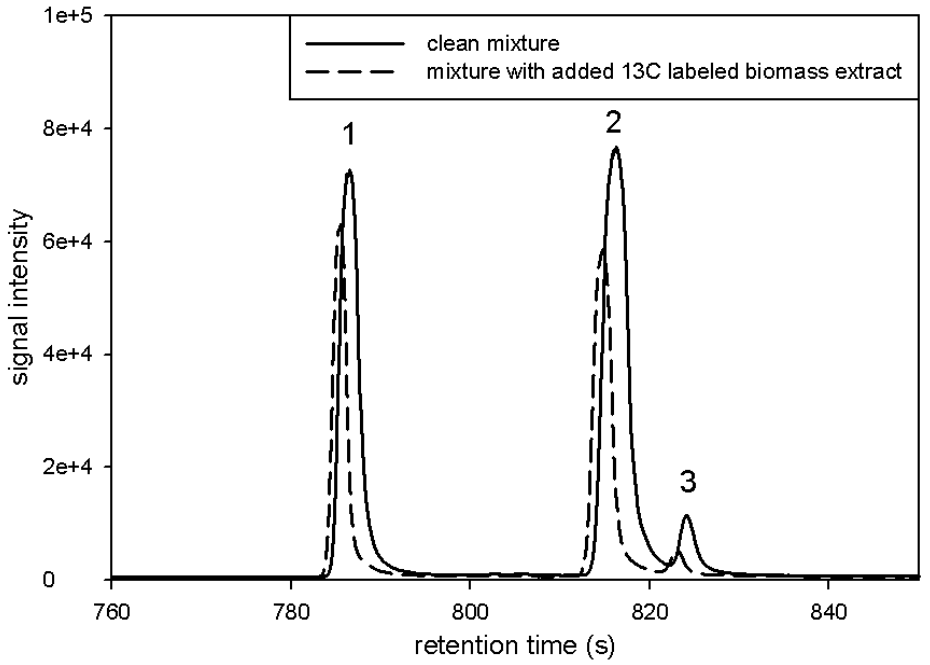
GC-TMS
hexoses
mannose (grey 1 + 4)
galactose (grey 2 + 5)
glucose (grey 3 + 5)
fructose (black 1 + 2)
myoinositole (RT = 645 s)

(grey 6 and black 3) =
glucuronic acid



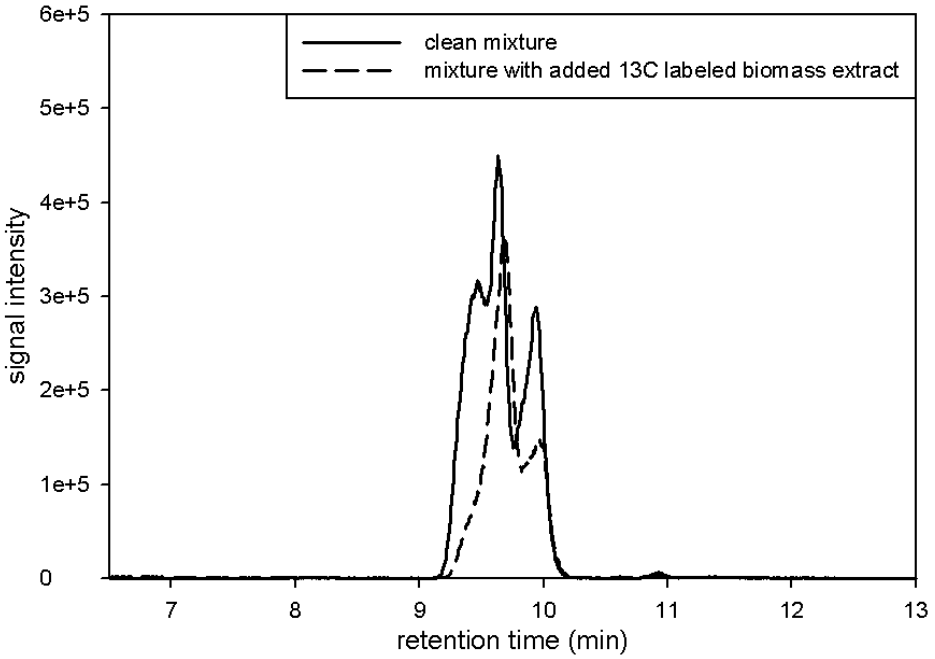
B

GC-TMS
disaccharides
(1) trehalose & sucrose
(2) & (3) maltose



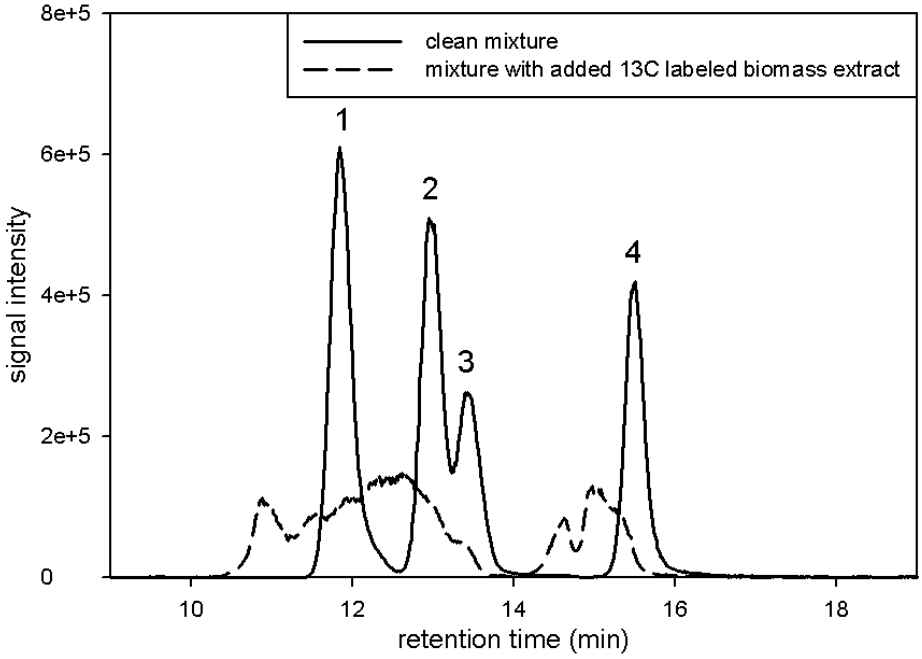
C

LC-HILIC
disaccharides
maltose
trehalose
sucrose



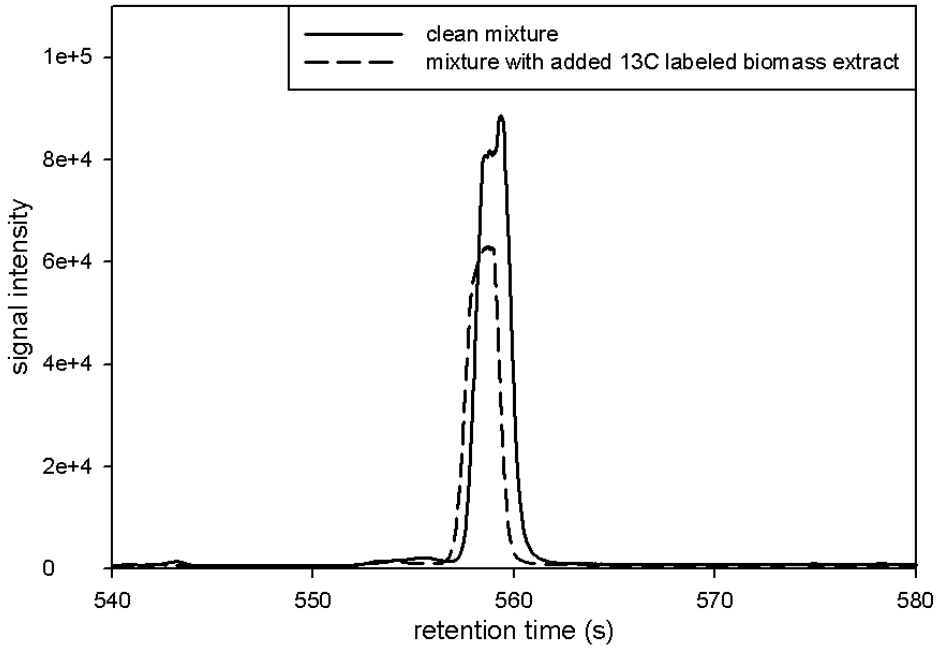
D

LC-ionpairing
hexose phosphates:
(1) glucose-6-P
(2) glucose-1-P
(3) fructose-6-P
(4) fructose-1-P



E

TMS
citrate & isocitrate



F

TBDMS
(1) citrate
(2) isocitrate