SERUM METABOLOMICS

Report on data description and quality control

(v3, 06/04/2017)

Dr. Alexandros P. Siskos
Dr. Chung-Ho E. Lau
Dr. Hector C. Keun
Dr. Muireann Coen

Imperial College London (UK)
Email: a.siskos@imperial.ac.uk

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1. Analytical Method

We have used the targeted metabolomics Absolute*IDQ*^{TM} p180 Kit (BIOCRATES Life Sciences AG). The kit allows the targeted analysis of 188 metabolites in the metabolite classes of amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingolipids and sum of hexoses, covering a wide range of analytes and metabolic pathways in one targeted assay. The Kit consists of a single sample processing procedure, although two separate MS analytical runs, using a combination of liquid chromatography (LC) and flow-injection analysis (FIA) coupled to tandem mass spectrometry (MS/MS). We have already carried out an inter-laboratory assessment of the kit, and demonstrated that the metabolite profiles produced are very reproducible and robust [Siskos et al, 2017].

Isotope-labelled and chemically homologous internal standards are used for quantification, and in total 54 analytes are fully validated as absolutely quantitative. Of the total 188 metabolites measured, 42 metabolites are measured by LC-MS/MS and 146 metabolites by FIA-MS/MS. The amino acids (21) and biogenic amines (21) are analysed quantitatively by LC-ESI-MS/MS, with the use of external calibration standards in seven different concentrations and isotope labelled internal standards for most analytes. All amino acids and amines are fully validated as absolutely quantitative. The acylcarnitines (40), glycerophospholipids (90), sphingolipids (15) and sum of hexoses (1) are analysed by FIA-ESI-MS/MS, using a one point internal standard calibration with representative internal standards (9 isotope-labelled acylcarnitines, 1 isotope-labelled hexose, 1 non-labelled lyso-PC, 2 non-labelled PCs, 1 non-labelled SM, a total of 14 internal standards). In terms of quantification, the lipids and a subset of acylcarnitines are called “semi-quantitative” since specific standards were not commercially available and a verification of the accuracy was not possible by the manufacturer. 11 acylcarnitines and the sum of hexoses are fully validated as absolutely quantitative. In addition many of the FIA-detected, semi-quantitative lipid concentrations represent total concentrations of possible isobars and structural isomers. The results for the metabolites are displayed with a corresponding short name with the total length of side chains and the total number of double bonds. The kit utilises a patented 96-well plate design which allows simultaneous efficient sample derivatisation and reproducible analyte extraction. The kit is suitable for manual or automated high throughput operation, and it requires only a very small sample volume of 10 µL and comes with human plasma based quality controls in 3 concentration levels (low, medium, high) which can be used for quality control purposes but also potentially for batch normalisation. Serum metabolic profiles were acquired on a Sciex QTrap 6500 equipped with an Agilent 1100 series HPLC, at Imperial College London (ICL), according to the manufacturer’s protocol [Biocrates Life Sciences AG, Innsbruck, Austria. User manual and Analytical specifications].

2. Sample description and batch design

We have acquired data for 1364 HELIX cohort samples in 18 batches. Batches of samples have been fully randomised to prevent potential analytical biases that would impact on subsequent data processing and statistical analysis. In every batch, which is in a 96-well plate format we have run:

- 4 x blanks
  - No sample, no IS (x1)
  - Zero (PBS) + IS (x3).
    - Used to calculated the LODs per metabolite, per batch
- 7 x calibration standards (LC-metabolites)
- 3 x kit QCs (low (x1), medium (x1), high (x1))
- 2 x CQC (SeraLab, S-123-M-27485)
- 4 x NIST – SRM 1950
- 76 x cohort samples, randomised

In every analytical batch, we run three sets of quality control samples, the NIST SRM 1950 (in 4 replicates), a commercial available serum QC material (CQC) and the QCs provided by the manufacturer in three concentration levels.

For batch 14, we run 72 cohort samples and 8 NIST – SRM 1950 samples. This was necessary as a new aliquot-batch of the reference material NIST SRM 1950 was prepared. To identify any differences between the two preparations (aliquots) of the reference material, we run 4 replicates per preparation (denoted as b1 and b2) in the same analytical batch (batch 14). The concentrations of most metabolites are essentially identical, except only putrescine, highlighting possibly poor long term stability of this specific compound.

3. Raw data transformation

For the LC-MS/MS assay, the metabolites were quantified by stable isotope dilution and seven point calibration curves. For the FIA-MS/MS assay, metabolite concentrations were calculated using a one point internal standard calibration, and are also isotope corrected. Metabolites were quantified (results shown in micromolar (μM) concentration units) according to the manufacturer's protocol using the Met*IDQ*™ Boron software for targeted metabolomic data processing and management. Blank PBS (phosphate-buffered saline) samples (3 replicates) were used for the calculation of the limits of detection (LOD). The median value of the PBS samples on the plate was calculated as approximation of the background noise per metabolite, and 3 times this value was calculated as the LOD, per metabolite, per batch. To avoid imputation during subsequent statistical analysis recorded values below the limits of detection (BLD) were not excluded from the analysis

The protocol used here consisted of a single sample processing procedure, however it requires two separate MS analytical runs, the LC-MS/MS and the FIA-MS/MS analysis of metabolites. LC offers the advantage of greater selectivity and lower susceptibility to matrix effects. However, for low intensity peaks (low concentration samples, PBS sample for calculation of LOD) visual inspection, optimisation of integration and manual integration may be necessary to get accurate results.

While the integration of peaks is unambiguous for any FIA detection method, variation in background, selectivity and matrix effects do remain and are likely to be a source of batch variability, which could be potentially necessary to normalise for.

4. Quality control – analytical performance

Excluded variables: Data for 188 metabolites were acquired with the LC/FIA-MS/MS targeted metabolomic protocol. 6 amines have been excluded, carnosine, DOPA, Dopamine, nitro-Tyr, c4-OH-pro, PEA. Visual inspection of raw data, showed there was no signal, because of very low abundance. Therefore, quality control and analytical performance was assessed for a total of 182 metabolites.

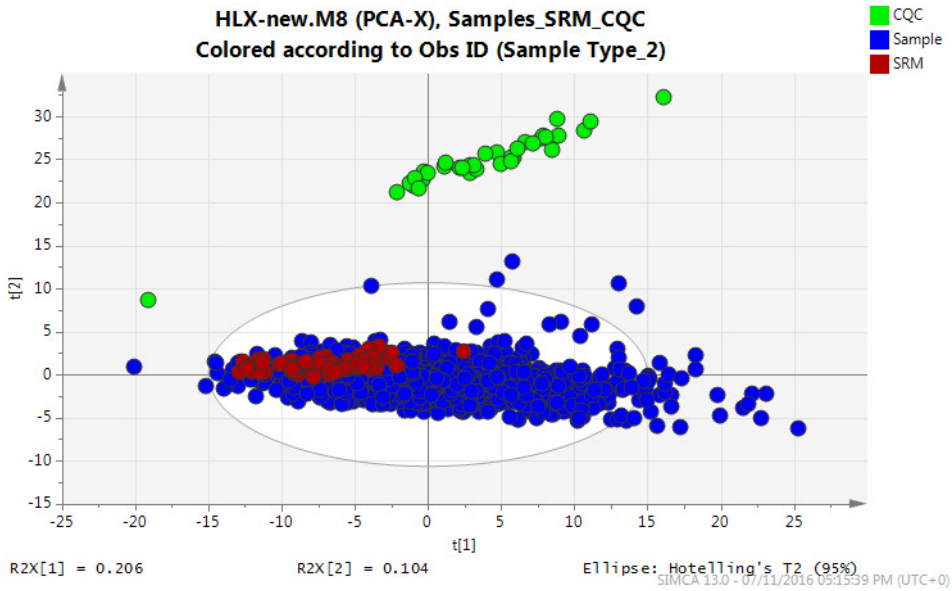

Figure 1. Score plots of the first two components of principal component analysis depicting the HELIX cohort samples and two of the QC material used the NIST SRM 1950 and the CQC. Data have not been normalised or scaled.

Principal Components Analysis was used to assess outlying samples with samples with T2Range > 99% identified as possible outliers. Two samples were identified as outliers, CQC2_160302_b2 (batch 4) and SAB-042-1x (batch 18). The sample SAB-042-1x was also noted that it was very haemolysed.

For the HELIX cohort samples and the NIST SRM 1950 (total 1440 samples), there are no missing values, but there are 47,939 values below the limit of detection (BLD) (18.3 %), of which 696 zero values (0.27 %). For the HELIX cohort samples only (1364 samples), there are 45,436 values below the limit of detection (BLD) (18.3 %), of which 679 zero values (0.27 %).

Table 1. Distribution of precision for the 182 detected metabolites, based on the % coefficient of variance for the analysis of 4 replicates of the SRM 1950 in the 18 analytical batches. Most metabolites (153, 84%) have %CV less than 20%.

CV	<10%	10-20%	20-30%	>30%	Total
LC-metabolites	21	9	2	4	36
FIA-metabolites	19	104	9	14	146
All metabolites	**40**	**113**	**11**	**18**	**182**

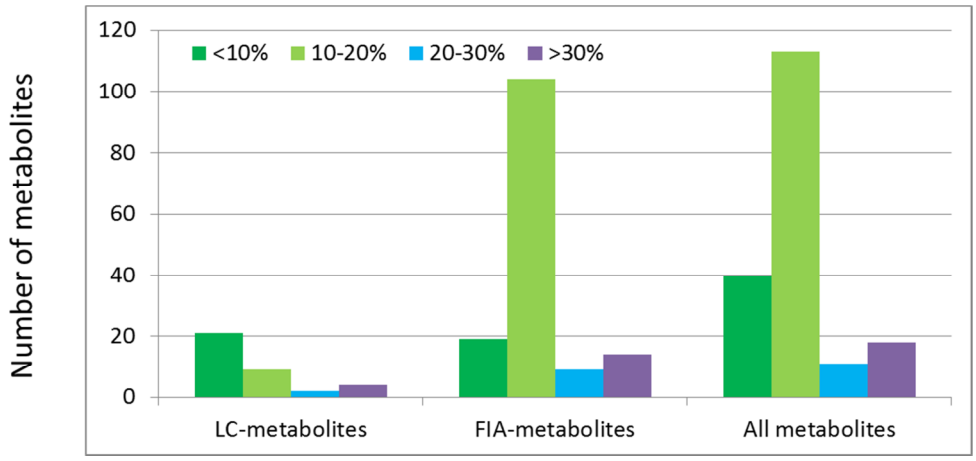

Figure 2. The figure shows the distribution of precision for the 182 detected metabolites, based on the % coefficient of variance for the analysis of 4 replicates of the SRM 1950 in the 18 analytical batches. Most metabolites (153, 84%) have %CV less than 20%.

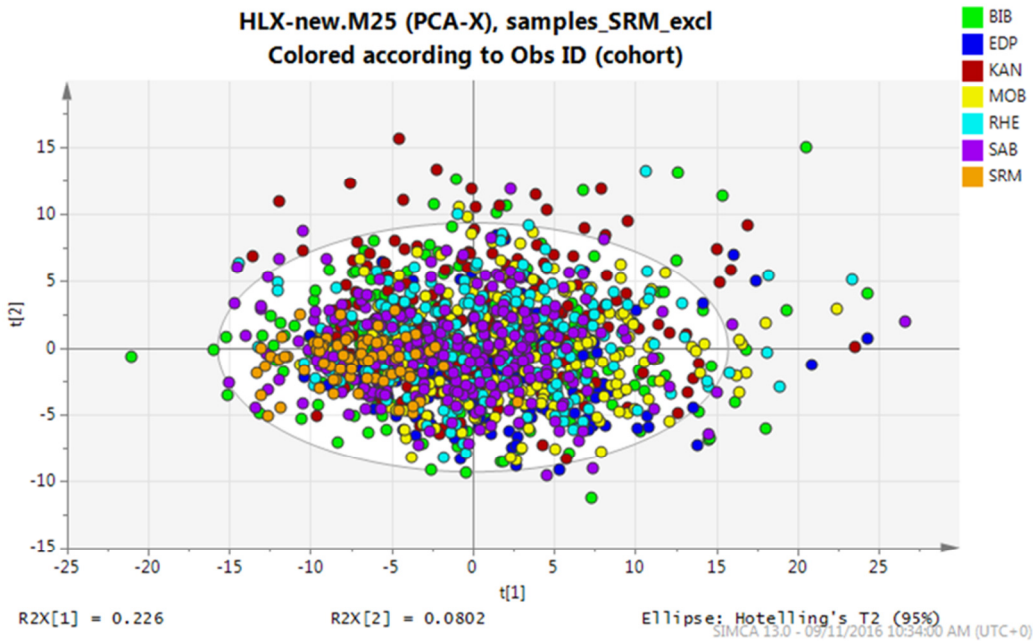

Figure 3. Score plots of the first two components of principal component analysis depicting Cohort effect to the serum metabolome. Figure also included the QC NIST SRM 1950 samples. Data have not been normalised or scaled.

Of the 182 detected metabolites we recommend the further exclusion from subsequent statistical analysis of the metabolites that meet both the following two criteria:
i) %CV > 30% **AND**
ii) % BLD+zeros >30 % (for the HELIX cohort samples).

These are 5 metabolites (Histamine, C3:1, C18:1-OH, PC aa C26:0, SM C22:3). Therefore, the final dataset of the serum metabolome consists of a total of 177 metabolites.

Conclusions: Most of the detected metabolites (153 of 182, 84%) have a %CV less than 20%. The data is therefore of satisfactory precision even if no normalisation has been applied to correct for any batch effect. 5 metabolites were excluded from further statistical analysis.

If during subsequent statistical data analysis batch normalisation is considered necessary, we recommend the use of a single normalisation factor for each metabolite and for each batch. This normalisation factor should be calculated using the SRM NIST 1950 reference material, as the ratio of the mean value for a measured metabolite in the reference material in all batches, divided by the mean (or median) value for the measured metabolite in the reference material in a specific batch. Further details on the normalisation can be found in [Siskos et al, 2017], or please contact the Imperial College team.

5. Data structure and transfer

The serum LC-MS metabolomic dataset is included in a single excel file ("serum_metabol_HELIX_IC_2017_01_27_v2") with 8 worksheets:

- 1st worksheet "Info": General info and variable description.
- 2nd worksheet "HLX_samples_SRM_177": Includes the **main dataset for 177 metabolite variables**, for a total of 1440 samples of which 1364 HELIX cohort samples, and 76 NIST-SRM 1950 QC samples (Table 2). Sample SAB-042-1x is included in the dataset; however it is a potential outlier. For convenience we have removed the Kit QCs, the CQC and the calibration curves blanks. The metabolite measurements are highlighted with colours that denote:
    - green: valid, fully quantitative and value is within the quantitation range.
    - light blue: quantitative and above the LOD, but value is <LLOQ or >ULOQ (LLOQ: lower limit of quantification, ULOQ: upper limit of quantification).
    - light green: semi-quantitative, and above the LOD, however LLOQ and ULOQ are not defined.
    - purple: <LOD (below limit of detection, BLD).
    - yellow: Some analytical calibration characteristics may be out of the expected range, but with no impact on the quality of data (this is useful as an operational warning to the experimental analyst, eg increased signals of blanks).
- 3rd worksheet "HLX_samples_SRM_ratios": **Custom calculated 42 metabolic indicators.** These are not the primary metabolomic dataset, but are variables calculated from the primary dataset of the 177 metabolites. They should not be the focus of the initial statistical analysis. We have calculated a set of specific metabolic ratios, sums and indicators that correspond to known biochemical and metabolic pathways, and have established biological significance. Please contact the Imperial College team for

more information on their significance and potential issues with quality control/statistical analysis etc.

- 4th worksheet "LODs_LOQs" : The **LODs** per metabolite, per analytical batch. Also for the metabolites that are validated as absolutely quantitative the LLOQ and ULOQ are provided.
- 5th worksheet "QC_performance" : **Analytical performance** (see next paragraph).
- 6th worksheet "Chebi": **Chebi metabolite annotations.**
- 7th worksheet "KEGG-LC": **KEGG metabolite annotations for aminoacids and biogenic amines only.**
- 8th worksheet "indicators_annotation": **Annotations for the custom metabolic indicators.** This includes a brief description with metabolic and biological significance, the formula by which these were calculated and also the identification of specific enzymes and pathways wherever possible.

For most of the 177 metabolites, more than 1 annotation is provided for either the Chebi of KEGG databases. This is to account for possible isomers. The annotation of the aminoacids and biogenic amines is almost definite for both databases and in most cases the possible database annotations are usually not more than 3 per measured metabolite. There is only one ambiguity with the KEGG annotations for SDMA and total DMA, where we used generic annotations that refer to the class of substrates / compounds.

For the lipids, carnitines and hexoses the annotations are accurate, to the best of our knowledge, however it is practically impossible to be an exhaustive and unambiguous list because of the presence of numerous possible isomers per measured molecule, and also because of unfortunately discrepancies and ambiguities in the databases for some of these molecules.

Table 2: Number of samples for serum LC-MS/MS metabolomics

Sample type	Number of samples
HELIX cohort samples	1364
HELIX main	1209
HELIX panel	155
NIST – SRM 1950 (QC)	76

6. List of reported variables and analytical performance

Table 3 below gives detailed data of all the quality control features per metabolite. The overall precision, based on the repeated measurements of the NIST SRM 1950, is provided as %CV and also the intra-batch and inter-batch precision. Moreover, the percentage of measurements below the limit of detection (including zero values) is provided for the 1364 HELIX cohort samples.

Table 3: Overall precision, intra-batch precision, inter-batch precision and % of values below the limit of detection per detected metabolite. The metabolite class and the detection assay (LC or FIA) are also denoted.

	Class	Class_2	Overal CV % (n=76)	intra-batch CV (mean) n=18 plates, n=4 replicates /plate	inter-batch CV % n=18 plates	% BLD + zeros (HELIX)
Ala	aminoacids	LC	7.5	7.0	3.4	0.0
Arg	aminoacids	LC	8.3	7.6	4.2	0.0
Asn	aminoacids	LC	8.1	7.6	3.7	0.0
Asp	aminoacids	LC	14.9	12.0	10.5	0.0
Cit	aminoacids	LC	10.2	8.4	5.4	0.0
Gln	aminoacids	LC	7.7	7.0	3.7	0.0
Glu	aminoacids	LC	9.0	8.4	4.0	0.0
Gly	aminoacids	LC	7.0	6.3	3.5	0.0
His	aminoacids	LC	7.8	7.2	3.6	0.0
Ile	aminoacids	LC	7.6	7.0	3.9	0.0
Leu	aminoacids	LC	7.8	7.1	3.8	0.0
Lys	aminoacids	LC	9.0	7.8	5.0	0.0
Met	aminoacids	LC	12.2	9.8	9.0	0.0
Orn	aminoacids	LC	8.6	7.2	4.2	0.0
Phe	aminoacids	LC	8.4	7.2	4.6	0.0
Pro	aminoacids	LC	7.9	6.9	4.3	0.0
Ser	aminoacids	LC	8.0	7.2	3.6	0.0
Thr	aminoacids	LC	8.0	7.5	3.4	0.0
Trp	aminoacids	LC	8.5	7.5	3.8	0.0
Tyr	aminoacids	LC	8.4	7.6	4.4	0.0
Val	aminoacids	LC	7.3	6.6	3.8	0.0
Ac-Orn	biogenic amines	LC	14.5	8.9	12.6	19.2
ADMA	biogenic amines	LC	11.8	11.3	5.1	0.0
alpha-AAA	biogenic amines	LC	21.8	10.5	20.7	5.6
Creatinine	biogenic amines	LC	7.5	6.9	3.2	0.0
Histamine	biogenic amines	LC	56.5	4.4	59.8	100.0
Kynurenine	biogenic amines	LC	9.6	8.9	4.1	0.0
Met-SO	biogenic amines	LC	16.4	9.9	14.5	4.8
Putrescine	biogenic amines	LC	44.8	34.5	37.0	5.1
SDMA	biogenic amines	LC	35.9	18.2	35.0	5.4
Serotonin	biogenic amines	LC	24.0	8.8	23.7	0.0
Spermidine	biogenic amines	LC	17.1	5.9	16.4	66.5
Spermine	biogenic amines	LC	36.6	28.1	33.3	3.0
t4-OH-Pro	biogenic amines	LC	10.0	7.3	7.4	0.0
Taurine	biogenic amines	LC	7.6	7.3	3.1	0.0
total DMA	biogenic amines	LC	14.1	8.3	12.2	0.0
C0	acylcarnitines	FIA	7.6	7.4	3.1	0.0
C10	acylcarnitines	FIA	8.6	7.8	4.6	16.9
C10:1	acylcarnitines	FIA	10.3	8.8	6.3	68.6

C10:2	acylcarnitines	FIA	8.8	7.5	5.4	99.8
C12	acylcarnitines	FIA	8.1	6.6	5.1	82.5
C12-DC	acylcarnitines	FIA	9.4	7.6	6.4	100.0
C12:1	acylcarnitines	FIA	10.1	8.8	6.3	71.3
C14	acylcarnitines	FIA	13.8	8.3	12.0	99.5
C14:1	acylcarnitines	FIA	11.9	11.4	5.1	1.2
C14:1-OH	acylcarnitines	FIA	13.4	13.0	6.2	98.8
C14:2	acylcarnitines	FIA	12.1	11.3	6.0	30.6
C14:2-OH	acylcarnitines	FIA	19.2	11.0	15.6	100.0
C16	acylcarnitines	FIA	10.4	9.9	5.1	0.1
C16-OH	acylcarnitines	FIA	24.0	8.7	21.0	100.0
C16:1	acylcarnitines	FIA	8.9	8.6	3.7	93.3
C16:1-OH	acylcarnitines	FIA	14.4	11.6	8.9	98.5
C16:2	acylcarnitines	FIA	22.5	11.6	21.0	99.4
C16:2-OH	acylcarnitines	FIA	11.9	11.0	6.5	100.0
C18	acylcarnitines	FIA	10.1	8.3	6.3	10.3
C18:1	acylcarnitines	FIA	9.0	9.0	3.4	30.9
C18:1-OH	acylcarnitines	FIA	56.7	7.9	54.6	100.0
C18:2	acylcarnitines	FIA	10.6	10.2	4.1	1.5
C2	acylcarnitines	FIA	7.4	7.0	3.3	0.0
C3	acylcarnitines	FIA	15.9	8.2	14.6	0.0
C3-DC (C4-OH)	acylcarnitines	FIA	17.4	6.5	16.9	67.2
C3-OH	acylcarnitines	FIA	29.4	18.3	23.8	99.9
C3:1	acylcarnitines	FIA	65.5	11.2	66.8	100.0
C4	acylcarnitines	FIA	16.5	8.8	15.1	0.0
C4:1	acylcarnitines	FIA	15.4	8.0	14.2	99.7
C6 (C4:1-DC)	acylcarnitines	FIA	10.1	6.9	8.0	79.8
C5	acylcarnitines	FIA	9.4	7.4	6.5	1.4
C5-M-DC	acylcarnitines	FIA	17.3	10.6	15.1	99.9
C5-OH (C3-DC-M)	acylcarnitines	FIA	8.7	6.9	6.0	100.0
C5:1	acylcarnitines	FIA	17.2	13.4	11.8	100.0
C5:1-DC	acylcarnitines	FIA	21.7	12.0	19.2	99.9
C5-DC (C6-OH)	acylcarnitines	FIA	17.7	10.3	15.2	98.5
C6:1	acylcarnitines	FIA	14.1	10.8	10.5	99.9
C7-DC	acylcarnitines	FIA	13.5	7.5	12.1	99.7
C8	acylcarnitines	FIA	10.7	7.8	8.3	82.7
C9	acylcarnitines	FIA	14.2	8.4	12.5	99.9
lysoPC a C14:0	glycerophospholipids	FIA	8.1	7.0	5.1	99.9
lysoPC a C16:0	glycerophospholipids	FIA	11.1	9.3	6.2	0.0
lysoPC a C16:1	glycerophospholipids	FIA	11.1	9.3	5.9	0.0
lysoPC a C17:0	glycerophospholipids	FIA	10.8	9.5	5.8	0.0
lysoPC a C18:0	glycerophospholipids	FIA	11.4	9.0	7.1	0.0
lysoPC a C18:1	glycerophospholipids	FIA	11.0	8.9	6.4	0.0
lysoPC a C18:2	glycerophospholipids	FIA	11.0	9.4	5.8	0.0
lysoPC a C20:3	glycerophospholipids	FIA	10.8	8.9	6.4	0.0

lysoPC a C20:4	glycerophospholipids	FIA	10.7	8.3	6.9	0.0
lysoPC a C24:0	glycerophospholipids	FIA	35.8	34.3	16.1	0.0
lysoPC a C26:0	glycerophospholipids	FIA	56.0	52.1	23.4	0.0
lysoPC a C26:1	glycerophospholipids	FIA	58.0	51.8	28.7	0.6
lysoPC a C28:0	glycerophospholipids	FIA	37.5	35.2	15.5	0.3
lysoPC a C28:1	glycerophospholipids	FIA	43.0	39.1	20.5	0.0
PC aa C24:0	glycerophospholipids	FIA	51.0	49.0	21.4	1.0
PC aa C26:0	glycerophospholipids	FIA	42.5	38.5	20.6	60.1
PC aa C28:1	glycerophospholipids	FIA	10.9	9.4	6.8	0.0
PC aa C30:0	glycerophospholipids	FIA	8.2	7.6	3.8	0.0
PC aa C30:2	glycerophospholipids	FIA	56.4	44.7	44.6	14.6
PC aa C32:0	glycerophospholipids	FIA	12.7	8.8	9.8	0.0
PC aa C32:1	glycerophospholipids	FIA	13.3	9.1	10.5	0.0
PC aa C32:2	glycerophospholipids	FIA	14.9	9.1	12.3	0.0
PC aa C32:3	glycerophospholipids	FIA	14.7	8.4	12.9	0.0
PC aa C34:1	glycerophospholipids	FIA	12.2	9.2	8.6	0.0
PC aa C34:2	glycerophospholipids	FIA	12.7	9.1	9.5	0.0
PC aa C34:3	glycerophospholipids	FIA	13.1	9.6	9.6	0.0
PC aa C34:4	glycerophospholipids	FIA	13.7	9.4	10.5	0.0
PC aa C36:0	glycerophospholipids	FIA	13.4	10.2	9.7	0.0
PC aa C36:1	glycerophospholipids	FIA	11.1	9.3	6.7	0.0
PC aa C36:2	glycerophospholipids	FIA	11.6	9.3	7.5	0.0
PC aa C36:3	glycerophospholipids	FIA	11.9	9.7	7.5	0.0
PC aa C36:4	glycerophospholipids	FIA	11.8	9.7	7.7	0.0
PC aa C36:5	glycerophospholipids	FIA	12.2	9.9	7.9	0.0
PC aa C36:6	glycerophospholipids	FIA	14.6	10.2	11.1	0.0
PC aa C38:0	glycerophospholipids	FIA	10.7	9.3	6.2	0.0
PC aa C38:1	glycerophospholipids	FIA	32.2	29.7	14.4	0.0
PC aa C38:3	glycerophospholipids	FIA	11.1	9.7	6.1	0.0
PC aa C38:4	glycerophospholipids	FIA	11.2	9.6	6.5	0.0
PC aa C38:5	glycerophospholipids	FIA	11.0	9.4	6.4	0.0
PC aa C38:6	glycerophospholipids	FIA	11.3	9.7	6.7	0.0
PC aa C40:1	glycerophospholipids	FIA	12.0	10.3	7.2	64.9
PC aa C40:2	glycerophospholipids	FIA	13.9	12.1	8.6	0.0
PC aa C40:3	glycerophospholipids	FIA	13.8	11.0	9.4	0.0
PC aa C40:4	glycerophospholipids	FIA	10.2	9.3	5.2	0.0
PC aa C40:5	glycerophospholipids	FIA	11.0	9.8	5.3	0.0
PC aa C40:6	glycerophospholipids	FIA	10.4	9.4	5.5	0.0
PC aa C42:0	glycerophospholipids	FIA	10.7	10.2	4.6	0.0
PC aa C42:1	glycerophospholipids	FIA	13.9	13.5	6.3	0.0
PC aa C42:2	glycerophospholipids	FIA	14.7	14.3	6.6	0.0
PC aa C42:4	glycerophospholipids	FIA	13.7	13.1	6.0	0.0
PC aa C42:5	glycerophospholipids	FIA	10.7	10.6	4.4	0.0
PC aa C42:6	glycerophospholipids	FIA	11.0	10.1	6.1	2.4
PC ae C30:0	glycerophospholipids	FIA	15.9	13.3	8.9	0.1

PC ae C30:1	glycerophospholipids	FIA	53.5	49.0	28.2	0.0
PC ae C30:2	glycerophospholipids	FIA	32.9	29.0	17.0	0.0
PC ae C32:1	glycerophospholipids	FIA	14.2	9.3	11.7	0.0
PC ae C32:2	glycerophospholipids	FIA	16.3	10.9	13.4	0.0
PC ae C34:0	glycerophospholipids	FIA	12.8	9.3	9.4	0.0
PC ae C34:1	glycerophospholipids	FIA	12.9	9.0	9.9	0.0
PC ae C34:2	glycerophospholipids	FIA	13.3	9.9	9.8	0.0
PC ae C34:3	glycerophospholipids	FIA	13.2	9.7	9.8	0.0
PC ae C36:0	glycerophospholipids	FIA	12.7	8.9	9.9	0.0
PC ae C36:1	glycerophospholipids	FIA	11.1	8.0	8.1	0.0
PC ae C36:2	glycerophospholipids	FIA	11.7	9.5	7.6	0.0
PC ae C36:3	glycerophospholipids	FIA	12.4	10.2	8.0	0.0
PC ae C36:4	glycerophospholipids	FIA	12.2	9.9	7.8	0.0
PC ae C36:5	glycerophospholipids	FIA	12.6	10.2	8.3	0.0
PC ae C38:0	glycerophospholipids	FIA	11.5	9.5	7.7	0.0
PC ae C38:1	glycerophospholipids	FIA	29.3	25.0	19.0	0.5
PC ae C38:2	glycerophospholipids	FIA	11.9	10.2	7.1	0.0
PC ae C38:3	glycerophospholipids	FIA	10.7	9.2	6.6	0.0
PC ae C38:4	glycerophospholipids	FIA	12.1	9.6	7.7	0.0
PC ae C38:5	glycerophospholipids	FIA	11.8	9.7	7.3	0.0
PC ae C38:6	glycerophospholipids	FIA	11.6	9.4	7.5	0.0
PC ae C40:1	glycerophospholipids	FIA	16.9	15.3	8.2	0.0
PC ae C40:2	glycerophospholipids	FIA	10.5	9.8	5.4	0.0
PC ae C40:3	glycerophospholipids	FIA	11.2	9.1	7.4	0.0
PC ae C40:4	glycerophospholipids	FIA	9.9	9.5	4.2	0.0
PC ae C40:5	glycerophospholipids	FIA	11.7	10.3	6.2	0.0
PC ae C40:6	glycerophospholipids	FIA	11.6	10.5	6.1	0.0
PC ae C42:0	glycerophospholipids	FIA	10.6	8.1	6.7	99.9
PC ae C42:1	glycerophospholipids	FIA	25.0	21.1	12.7	10.3
PC ae C42:2	glycerophospholipids	FIA	16.0	13.0	9.3	0.0
PC ae C42:3	glycerophospholipids	FIA	15.0	13.4	6.7	0.0
PC ae C42:4	glycerophospholipids	FIA	10.3	9.9	4.4	0.0
PC ae C42:5	glycerophospholipids	FIA	9.0	8.6	3.9	0.0
PC ae C44:3	glycerophospholipids	FIA	28.3	24.3	15.8	0.0
PC ae C44:4	glycerophospholipids	FIA	13.7	12.0	7.5	0.0
PC ae C44:5	glycerophospholipids	FIA	11.1	10.5	5.2	0.0
PC ae C44:6	glycerophospholipids	FIA	10.2	9.4	4.1	0.0
SM (OH) C14:1	sphingolipids	FIA	9.1	7.5	5.5	0.0
SM (OH) C16:1	sphingolipids	FIA	10.0	7.6	7.0	0.0
SM (OH) C22:1	sphingolipids	FIA	15.4	7.9	13.5	0.0
SM (OH) C22:2	Sphingolipids	FIA	15.6	8.6	13.3	0.0
SM (OH) C24:1	Sphingolipids	FIA	16.2	10.5	13.3	0.0
SM C16:0	Sphingolipids	FIA	9.1	7.5	5.7	0.0
SM C16:1	Sphingolipids	FIA	9.3	7.2	5.9	0.0
SM C18:0	Sphingolipids	FIA	10.9	7.1	8.6	0.0

SM C18:1	Sphingolipids	FIA	10.3	7.7	7.2	0.0
SM C20:2	Sphingolipids	FIA	13.9	11.7	9.3	0.0
SM C22:3	Sphingolipids	FIA	62.9	51.9	46.9	35.0
SM C24:0	Sphingolipids	FIA	15.3	8.0	13.4	0.0
SM C24:1	Sphingolipids	FIA	16.4	9.0	13.9	0.0
SM C26:0	Sphingolipids	FIA	27.4	22.6	17.5	0.0
SM C26:1	Sphingolipids	FIA	27.1	21.2	18.5	0.0
H1	Sugars	FIA	7.2	6.9	2.8	0.0

References:

Siskos AP, Jain P, Römisch-Margl W, Bennett M, Achaintre D, Asad Y, Marney L, Richardson L, Koulman A, Griffin JL, Raynaud F, Scalbert A, Adamski J, Prehn C, Keun HC, **2017**. Interlaboratory Reproducibility of a Targeted Metabolomics Platform for Analysis of Human Serum and Plasma. ***Anal Chem.*** 89(1), 656-665.

Biocrates Life Sciences AG, Innsbruck, Austria. User manual: UM_p180_AB SCIEX_9; 2014, Analytical specifications: AS_p180_4; 2014.