SERUM METABOLOMICS

Data description

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1. Introduction

We have used the targeted metabolomics AbsoluteIDQ^{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.

3. Data structure and transfer

The serum LC-MS metabolomic dataset is included in a single excel file with 5 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. 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)

List of reported variables and analytical performance

Table 2 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 1440 samples (1364 cohort samples and 76 SRM 1950).

Table 2: 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	
			Overall total CV % (n=76)	intra-batch CV % (mean)	inter-batch CV %	% BLD + zeros
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	18.9
ADMA	biogenic amines	LC	11.8	11.3	5.1	0.0
alpha-AAA	biogenic amines	LC	21.8	10.5	20.7	6.0

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	5.3
Putrescine	biogenic amines	LC	44.8	34.5	37.0	5.8
SDMA	biogenic amines	LC	35.9	18.2	35.0	5.5
Serotonin	biogenic amines	LC	24.0	8.8	23.7	0.0
Spermidine	biogenic amines	LC	17.1	5.9	16.4	66.3
Spermine	biogenic amines	LC	36.6	28.1	33.3	3.3
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	16.3
C10:1	acylcarnitines	FIA	10.3	8.8	6.3	68.7
C10:2	acylcarnitines	FIA	8.8	7.5	5.4	99.8
C12	acylcarnitines	FIA	8.1	6.6	5.1	83.4
C12-DC	acylcarnitines	FIA	9.4	7.6	6.4	100.0
C12:1	acylcarnitines	FIA	10.1	8.8	6.3	72.1
C14	acylcarnitines	FIA	13.8	8.3	12.0	99.5
C14:1	acylcarnitines	FIA	11.9	11.4	5.1	1.1
C14:1-OH	acylcarnitines	FIA	13.4	13.0	6.2	98.9
C14:2	acylcarnitines	FIA	12.1	11.3	6.0	29.1
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	93.6
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	29.7
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	64.0
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	80.8
C5	acylcarnitines	FIA	9.4	7.4	6.5	1.3
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	98.6
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	83.4
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	60.2
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	14.0
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	66.5
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	9.9
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	33.8
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:

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