应用纪要

Standardized Targeted Metabolomics Using the BIOCRATES MxP Quant 500 Kit on the ACQUITY UPLC I-Class PLUS and Xevo TQ-XS Mass Spectrometer

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Abstract

In this application note, we present the validation of the quantitative MxP Quant 500 kit on a Waters Xevo TQ-XS Mass Spectrometer. The assay offers multiplexed MS/MS analysis of up to 630 metabolites and lipids from 26 analyte classes in only a 10-µL sample volume. In addition, 232 pre-defined metabolism indicators, that are biologically meaningful sums and ratios of metabolites and lipids, can be calculated by the BIOCRATES MetIDQ software tool, MetaboINDICATOR. Combined, these are 852 metabolic features that could be determined in one experiment with the MxP Quant 500 kit.

Benefits

- Ready-to-use BIOCRATES quantitative metabolomics kit solution including reagents and software
- Broad coverage of multiple metabolic pathways

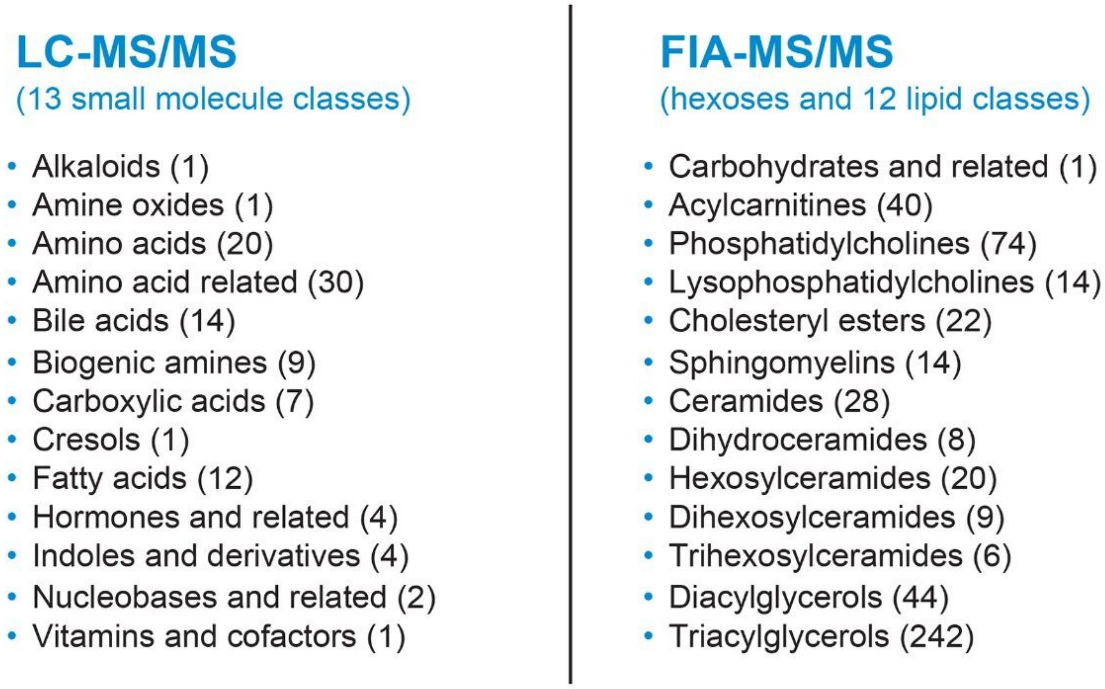
- Validated across multiple sample types

Introduction

Metabolic signatures can provide crucial insights into physiological mechanisms, as well as a better understanding of diseases. For comprehensive metabolomics and lipidomics analyses, analytical reliability, inter-laboratory comparability, automation, and standardization are of utmost importance.

Here, we present the validation of the quantitative MxP Quant 500 kit on a Waters Xevo TQ-XS Mass Spectrometer. The assay offers multiplexed MS/MS analysis of up to 630 metabolites and lipids from 26 analyte classes in only a 10-µL sample volume. In addition, 232 pre-defined metabolism indicators, that are biologically meaningful sums and ratios of metabolites and lipids, can be calculated by the BIOCRATES MetIDQ software tool, MetaboINDICATOR. Combined, these are 852 metabolic features that could be determined in one experiment with the MxP Quant 500 kit.

While the assay has been initially validated for human plasma, it also allows standardized analysis in a variety of biological sample matrices (e.g., blood, feces, and tissue) and species, including studies of the gut microbiome. The MxP Quant 500 kit combines LC-MS/MS of 13 compound classes, basically small molecules, bile acids, and free fatty acids, followed by FIA-MS/MS of 12 lipid classes (including acylcarnitines) and hexoses, into a single workflow (Figure 1).



LC-MS/MS
(13 small molecule classes)

- Alkaloids (1)
- Amine oxides (1)
- Amino acids (20)
- Amino acid related (30)
- Bile acids (14)
- Biogenic amines (9)
- Carboxylic acids (7)
- Cresols (1)
- Fatty acids (12)
- Hormones and related (4)
- Indoles and derivatives (4)
- Nucleobases and related (2)
- Vitamins and cofactors (1)

FIA-MS/MS
(hexoses and 12 lipid classes)

- Carbohydrates and related (1)
- Acylcarnitines (40)
- Phosphatidylcholines (74)
- Lysophosphatidylcholines (14)
- Cholesteryl esters (22)
- Sphingomyelins (14)
- Ceramides (28)
- Dihydroceramides (8)
- Hexosylceramides (20)
- Dihexosylceramides (9)
- Trihexosylceramides (6)
- Diacylglycerols (44)
- Triacylglycerols (242)

Figure 1. The MxP Quant 500 metabolite panel.

The Xevo TQ-XS Mass Spectrometer offers a broader dynamic range and increased sensitivity compared to its predecessors. Analytical validation was performed on a Waters ACQUITY UPLC I-Class PLUS System coupled to a Xevo TQ-XS Mass Spectrometer for human plasma, following EMA and FDA guidelines.

Experimental

High throughput analysis with a minimal sample volume (10 µL) is achieved by an easy and rapid sample preparation using a patented 96-well filter plate as described in the MxP Quant 500 kit user manual. Blank and zero samples, seven calibration standards, three levels of quality control samples (human plasma-based QCs), and a variety of plasma samples were subjected to two UPLC-ESI-MS/MS analyses in multiple reaction monitoring (MRM) mode, followed by two FIA-MS/MS runs. A Waters

ACQUITY UPLC I-Class PLUS System, equipped with a reversed-phase MxP Quant 500 UHPLC Column, was coupled to a Xevo TQ-XS Mass Spectrometer. Figure 2 shows two representative chromatograms (total ion current (TIC), positive, and negative ion modes) for a human plasma sample.

For quantitation, both LC and FIA data were converted and imported directly into the BIOCRATES software, MetIDQ Oxygen, and quantified. MetIDQ includes an automated simple target normalization procedure based on QC or sample pool for batch-to-batch and kit plate-to-plate correction for sample cohort across several kit plates.

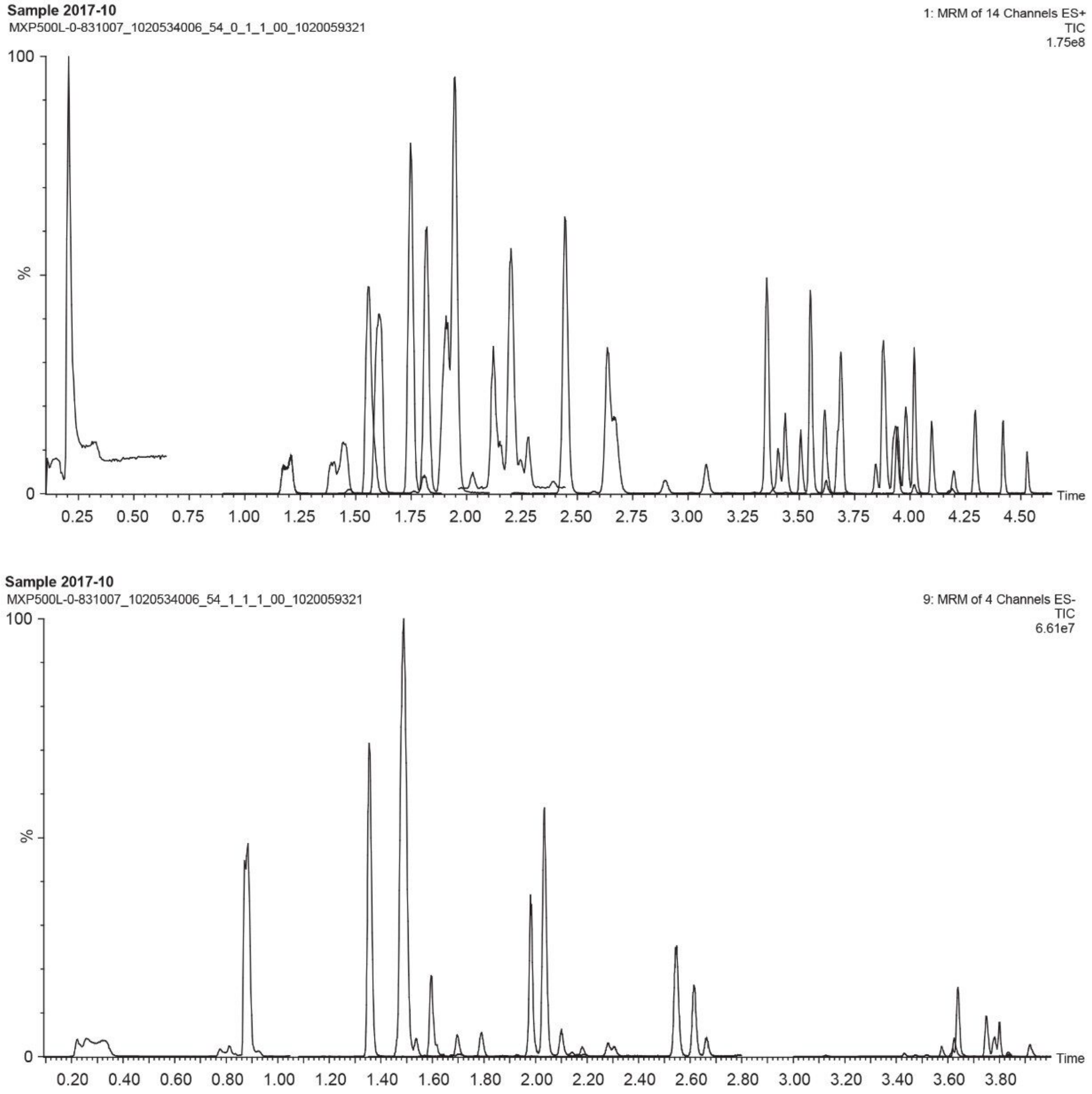


Figure 2. LC chromatograms (total ion current (TIC), segmented) derived from a human plasma sample in positive (upper panel) and negative (lower panel) ion modes measured with the BIOCRATES MxP Quant 500 kit on Waters ACQUITY UPLC I-Class PLUS System and Xevo TQ-XS.

MxP Quant 500 kit

The MxP Quant 500 kit is a ready-to-use kit for standardized, multiplexed MS/MS analysis of up to 630 metabolites and lipids. It includes an automated software guided workflow from sample registration to data analysis. The kit is equipped with hardware (patented kit plate, reagents, additives to mobile phase, column set, and USB stick with predefined read-to-use FIA-MS/MS and UHPLC-MS/MS analysis and quantitation methods validated for Xevo TQ-XS) and software (MetIDQ).

Sample preparation

Sample preparation was performed according to the BIOCRATES MxP Quant 500 kit user manual. In brief, 10 µL of sample (blood plasma, serum, tissue, and fecal homogenate), blank, zero sample, kit calibrator, and kit quality control material were each added directly onto the 96-well plate provided with the kit according to the pipetting plan predefined in MetIDQ. After a drying step of 30 min using nitrogen, a derivatization step for one hour using a 5% PITC solution was performed. After another drying step of one hour, 300 µL of 5 mM methanolic ammonium acetate were added as extraction solvent and, after shaking of the kit plate for 30 min, the contents were filtered into a lower sandwich plate by centrifugation at 200 g for 2 min. The sample extracts were diluted for subsequent FIA-MS/MS and UHPLC-MS/MS analysis as specified in the kit user manual.

Data management

MassLynx v4.2 Software with MetIDQ Software

UHPLC system conditions

System:	ACQUITY UPLC I-Class PLUS
Needle:	20 µL
Column:	BIOCRATES MxP Quant 500 column (part of kit)
Weak wash solvent:	Methanol/water 1:3
Strong wash solvent:	Acetonitrile/methanol/isopropanol/ water 5:2:1.5:1.5
Column temp.:	50 °C

MS system conditions

System:	Xevo TQ-XS
Acquisition mode:	Multiple Reaction Monitoring (MRM)
Polarity:	LC 1 (ESI+), LC 2 (ESI-), FIA 1 (ESI+), FIA 2 (ESI+)
Capillary:	4 kV, 2 kV, 3.32 kV, 3.6 kV
Source temp.:	150 °C, 150 °C, 150 °C, 150 °C
Desolvation temp.:	600 °C, 525 °C, 500 °C, 500 °C
Inter-scan delay:	0.003 s
Inter-channel delay:	0.003 s

Results and Discussion

The adaption of the ready-to-use MxP Quant 500 kit to the Xevo TQ-XS LC-MS/MS system comprised the optimization of instrumental parameters from sample preparation setup to mass spectrometric features.

Overall, analysis times were 7 min per LC-MS/MS run and 4 min per FIA-MS/MS run, resulting in a total analysis run time of 36 hours for 80 samples plus quality control samples, calibration standards, a blank, and zero samples on a 96-well plate. Overall the kit run time from sample preparation to data processing and technical validation is about 42 hours.

Validation: Summary

A comprehensive set of defined human plasma samples was used for the validation of the MxP Quant 500 kit on the Waters ACQUITY UPLC I-Class PLUS System coupled to a Xevo TQ-XS Mass Spectrometer. Appendix 1 provides an overview of the analytical performance (classification) of the LC-MS/MS analytes. To determine the validity of the analytes, intra-day (within batch) and inter-day (batch-to-batch) analyses were evaluated in terms of precision and accuracy, as well as detection sensitivity, selectivity, and matrix effects. Excellent intra- and inter-batch accuracy (between 85–115%) and coefficient of variation as a measure for precision (CV <15%) were obtained for all seven-point calibrated analytes and their calibrants above the limit of detection (LOD) in the course of the validation, therefore classified as “quantitative”.

LC analytes that rely on a one-point internal calibration were accepted at a CV <20% and an accuracy between 80–120%, therefore classified as “quantitative with restriction”. If the accuracy criteria were not fulfilled, they were classified as “relative quantitative”. Two analytes could not be validated because many of their measured values were <LOD. No analytes were classified as “invalid”.

The NIST standard reference material (SRM) 1950 was analyzed and the measured concentration values were compared to the certified values to demonstrate the performance of the MxP Quant 500 kit on the Xevo TQ-XS system. Excellent accuracy between 85–115% was obtained for specified analytes in the reference material, amino acids, creatinine, and hexoses (Figure 3). These findings are important in the context of inter-laboratory comparability.

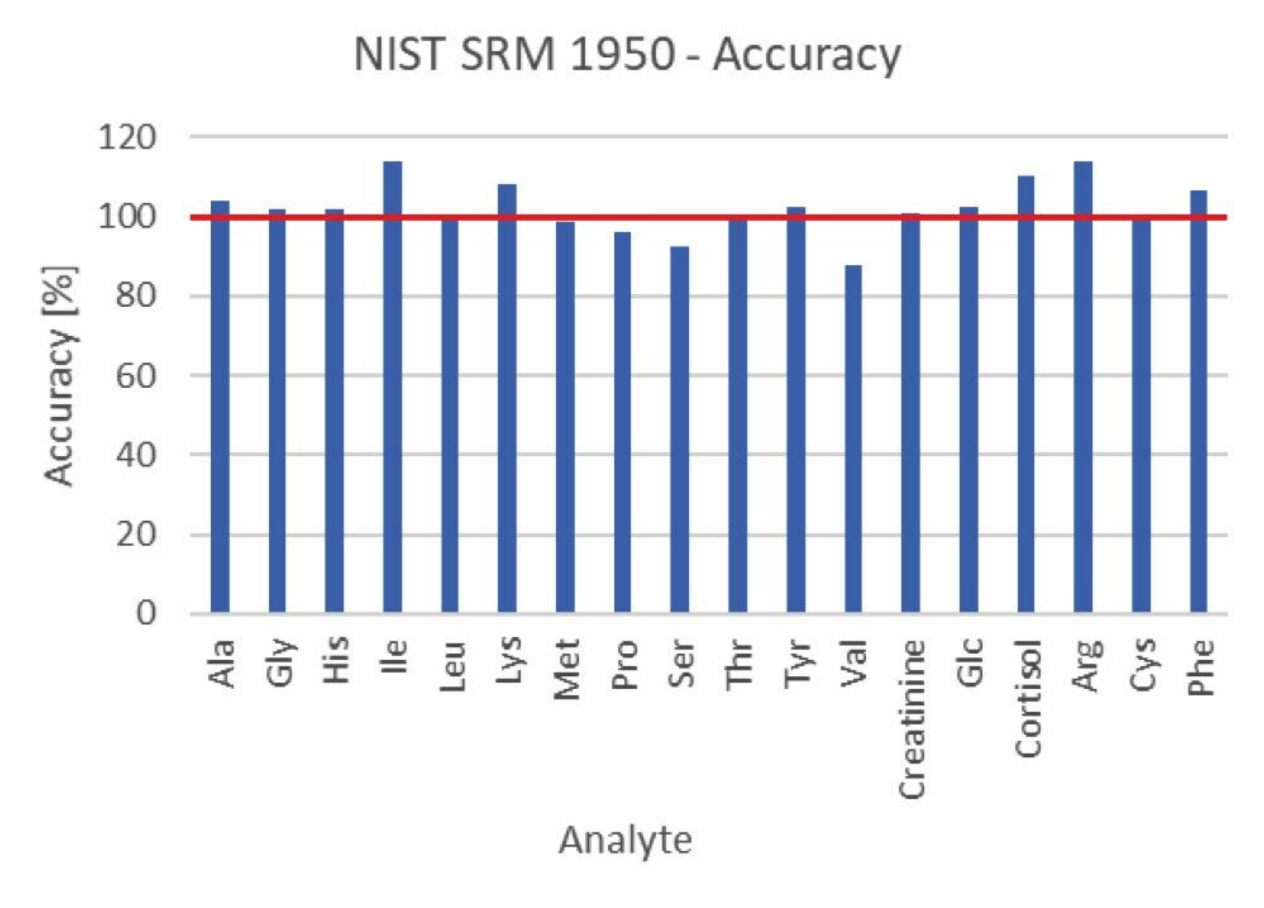


Figure 3. Accuracy of measurements compared to certified NIST SRM 1950 values (n=3). Note: Arg, Cys, and Phe are not-certified reference values.

Furthermore, a broad spectrum of metabolites, mostly lipids, present in biological specimens can be determined by FIA-MS/MS analysis with the MxP Quant 500 kit. These metabolites belong to key analyte classes, such as acylcarnitines (including carnitine), carbohydrates (hexoses), and a number of lipid classes (Figure 1). An overview of the analytical performance classification of acylcarnitines in human plasma is provided in Appendix 2. For FIA-MS/MS, the label quantitative implies precision <15% and accuracy between 80–120%. Since not all FIA-MS/MS metabolites are commercially available as external and internal standards, the accurate determination of lipids and a subset of acylcarnitines is limited. Thus, the validity of these analytes is termed “relative quantitative”. The label <LOD means that analytes could not be validated in human plasma because in most cases the measured concentrations were below the detection limits. In a typical human plasma sample approximately 520 smallmolecules and lipids could be routinely analyzed (>LOD) and quantified with the MxP Quant 500 kit. Excellent precision values were obtained for the vast majority of metabolites, making them highly suited for comparative studies of plasma from different sample cohorts.

In addition, the MxP Quant 500 kit on the Xevo TQ-XS Mass Spectrometer was also evaluated for rat plasma, mouse liver homogenate, and human fecal samples. The latter are particularly relevant for microbiome research. In these experiments >220 analytes were detected in a human fecal pool with CVs <20%, among these were acylcarnitines C3:1, C5:1, and C6:1, which are <LOD in plasma samples. Note that there is a separate application note detailing a recommended sample preparation protocol for the analysis of fecal samples with the MxP Quant 500 kit.

Comparison of Xevo TQ-XS and TQ-S

The analytical performance of the MxP Quant 500 kit on the ACQUITY UPLC I-Class PLUS System coupled to a Xevo TQ-XS Mass Spectrometer was compared to their performance on a Xevo TQ-S Mass Spectrometer.

The advantages of the TQ-XS are:

- An improved instrument sensitivity
- Related, shorter necessary dwell times
- A broader dynamic range

This results in approximately 20 analytes more being detectable using the Xevo TQ-XS. The broader dynamic range improves the linearity especially of higher abundant metabolites. The number of FIA injections could be reduced from three to two, resulting in a total instrument run time of about 33 instead of 40 hours per 96-well plate.

Conclusion

The data shown in this application note supports the high reliability and analytical potency of the MxP Quant 500 kit on the ACQUITY UPLC I-Class PLUS, Xevo TQ-XS system, delivering high level of global standardization in metabolomics with excellent lab-to-lab comparability.

Appendix 1

Metabolite	Metabolite Class	Classification
Trigonelline	Alkaloids	QR
TMAO	Amine oxides	RQ
Ala	Amino acids	Q
Arg	Amino acids	Q
Asn	Amino acids	Q
Asp	Amino acids	Q
Cys	Amino acids	QR
Gln	Amino acids	Q
Glu	Amino acids	Q
Gly	Amino acids	Q
His	Amino acids	Q
Ile	Amino acids	Q
Leu	Amino acids	Q
Lys	Amino acids	Q
Met	Amino acids	Q
Phe	Amino acids	Q
Pro	Amino acids	Q
Ser	Amino acids	Q
Thr	Amino acids	Q
Trp	Amino acids	Q
Tyr	Amino acids	Q
Val	Amino acids	Q
1-Met-His	Amino acid related	QR
3-Met-His	Amino acid related	QR
5-AVA	Amino acid related	QR
AABA	Amino acid related	QR
Ac-Orn	Amino acid related	Q
ADMA	Amino acid related	Q
alpha-AAA	Amino acid related	Q
Anserine	Amino acid related	QR
BABA	Amino acid related	QR
Betaine	Amino acid related	QR
c4-OH-Pro	Amino acid related	Q
Carnosine	Amino acid related	Q
Cit	Amino acid related	Q
Creatinine	Amino acid related	Q
Cystine	Amino acid related	QR
DOPA	Amino acid related	Q
HArg	Amino acid related	QR
HCys	Amino acid related	QR
Kynurenine	Amino acid related	Q
Met-SO	Amino acid related	Q
Nitro-Tyr	Amino acid related	Q
Orn	Amino acid related	Q
PAG	Amino acid related	QR
PheAlaBetaine	Amino acid related	QR
ProBetaine	Amino acid related	QR
Sarcosine	Amino acid related	Q
SDMA	Amino acid related	Q
t4-OH-Pro	Amino acid related	Q
Taurine	Amino acid related	Q
TrpBetaine	Amino acid related	QR
CA	Bile acids	QR

Metabolite	Metabolite Class	Classification
CDCA	Bile acids	QR
DCA	Bile acids	QR
GCA	Bile acids	QR
GCDCA	Bile acids	QR
GDCA	Bile acids	QR
GLCA	Bile acids	QR
GLCAS	Bile acids	QR
GUDCA	Bile acids	QR
TCA	Bile acids	QR
TCDCA	Bile acids	QR
TDCA	Bile acids	QR
TLCA	Bile acids	QR
TMCA	Bile acids	QR
beta-Ala	Biogenic amines	QR
Dopamine	Biogenic amines	Q
GABA	Biogenic amines	QR
Histamine	Biogenic amines	Q
PEA	Biogenic amines	Q
Putrescine	Biogenic amines	Q
Serotonin	Biogenic amines	Q
Spermidine	Biogenic amines	Q
Spermine	Biogenic amines	Q
AconAcid	Carboxylic acids	QR
DiCA(12:0)	Carboxylic acids	QR
DiCA(14:0)	Carboxylic acids	QR
HipAcid	Carboxylic acids	QR
Lac	Carboxylic acids	QR
OH-GlutAcid	Carboxylic acids	QR
Suc	Carboxylic acids	QR
p-Cresol-SO4	Cresols	QR
AA	Fatty acids	QR
DHA	Fatty acids	QR
EPA	Fatty acids	QR
FA(12:0)	Fatty acids	RQ
FA(14:0)	Fatty acids	RQ
FA(16:0)	Fatty acids	<LOD
FA(18:0)	Fatty acids	<LOD
FA(18:1)	Fatty acids	QR
FA(18:2)	Fatty acids	RQ
FA(20:1)	Fatty acids	RQ
FA(20:2)	Fatty acids	RQ
FA(20:3)	Fatty acids	RQ
AbsAcid	Hormones and related	QR
Cortisol	Hormones and related	QR
Cortisone	Hormones and related	QR
DHEAS	Hormones and related	QR
3-IAA	Indoles and derivatives	QR
3-IPA	Indoles and derivatives	QR
Ind-SO4	Indoles and derivatives	QR
Indole	Indoles and derivatives	QR
Hypoxanthine	Nucleobases and related	QR
Xanthine	Nucleobases and related	QR
Choline	Vitamins and cofactors	QR

Analyte classes and classification of analytical performance with LC-MS/MS.

Abbreviations: LOD, limit of detection; Q, quantitative; QR, quantitative with restrictions; and RQ, relative *quantitative.*

Appendix 2

Metabolite	Metabolite class	Analyte classification
C0	Acylcarnitines	Q
C2	Acylcarnitines	Q
C3	Acylcarnitines	Q
C3-DC (C4-OH)	Acylcarnitines	RQ
C3-OH	Acylcarnitines	<LOD
C3:1	Acylcarnitines	<LOD
C4	Acylcarnitines	Q
C4:1	Acylcarnitines	<LOD
C5	Acylcarnitines	Q
C5-DC (C6-OH)	Acylcarnitines	<LOD
C5-M-DC	Acylcarnitines	<LOD
C5-OH (C3-DC-M)	Acylcarnitines	RQ
C5:1	Acylcarnitines	<LOD
C5:1-DC	Acylcarnitines	<LOD
C6 (C4:1-DC)	Acylcarnitines	Q
C6:1	Acylcarnitines	<LOD
C7-DC	Acylcarnitines	RQ
C8	Acylcarnitines	Q
C9	Acylcarnitines	RQ
C10	Acylcarnitines	Q
C10:1	Acylcarnitines	<LOD
C10:2	Acylcarnitines	<LOD
C12	Acylcarnitines	Q
C12-DC	Acylcarnitines	<LOD
C12:1	Acylcarnitines	<LOD
C14	Acylcarnitines	Q
C14:1	Acylcarnitines	RQ
C14:1-OH	Acylcarnitines	RQ
C14:2	Acylcarnitines	RQ
C14:2-OH	Acylcarnitines	<LOD
C16	Acylcarnitines	Q
C16-OH	Acylcarnitines	<LOD
C16:1	Acylcarnitines	RQ
C16:1-OH	Acylcarnitines	RQ
C16:2	Acylcarnitines	<LOD
C16:2-OH	Acylcarnitines	<LOD
C18	Acylcarnitines	Q
C18:1	Acylcarnitines	RQ
C18:1-OH	Acylcarnitines	<LOD
C18:2	Acylcarnitines	RQ
lysoPC a C14:0	Glycerophospholipids	<LOD
lysoPC a C16:0	Glycerophospholipids	RQ
lysoPC a C16:1	Glycerophospholipids	RQ
lysoPC a C17:0	Glycerophospholipids	RQ
lysoPC a C18:0	Glycerophospholipids	RQ
lysoPC a C18:1	Glycerophospholipids	RQ
lysoPC a C18:2	Glycerophospholipids	RQ
lysoPC a C20:3	Glycerophospholipids	RQ
lysoPC a C20:4	Glycerophospholipids	RQ
lysoPC a C24:0	Glycerophospholipids	RQ
lysoPC a C26:0	Glycerophospholipids	RQ
lysoPC a C26:1	Glycerophospholipids	RQ
lysoPC a C28:0	Glycerophospholipids	RQ
lysoPC a C28:1	Glycerophospholipids	RQ
PC aa C24:0	Glycerophospholipids	RQ
PC aa C26:0	Glycerophospholipids	<LOD
PC aa C28:1	Glycerophospholipids	RQ
PC aa C30:0	Glycerophospholipids	RQ
PC aa C32:0	Glycerophospholipids	RQ
PC aa C32:1	Glycerophospholipids	RQ
PC aa C32:2	Glycerophospholipids	RQ
PC aa C32:3	Glycerophospholipids	RQ
PC aa C34:1	Glycerophospholipids	RQ
PC aa C34:2	Glycerophospholipids	RQ
PC aa C34:3	Glycerophospholipids	RQ
PC aa C34:4	Glycerophospholipids	RQ
PC aa C36:0	Glycerophospholipids	RQ
PC aa C36:1	Glycerophospholipids	RQ
PC aa C36:2	Glycerophospholipids	RQ
PC aa C36:3	Glycerophospholipids	RQ
PC aa C36:4	Glycerophospholipids	RQ
PC aa C36:5	Glycerophospholipids	RQ
PC aa C36:6	Glycerophospholipids	RQ
PC aa C38:0	Glycerophospholipids	RQ
PC aa C38:3	Glycerophospholipids	RQ
PC aa C38:4	Glycerophospholipids	RQ
PC aa C38:5	Glycerophospholipids	RQ
PC aa C38:6	Glycerophospholipids	RQ
PC aa C40:1	Glycerophospholipids	RQ
PC aa C40:2	Glycerophospholipids	RQ
PC aa C40:3	Glycerophospholipids	RQ
PC aa C40:4	Glycerophospholipids	RQ
PC aa C40:5	Glycerophospholipids	RQ
PC aa C40:6	Glycerophospholipids	RQ
PC aa C42:0	Glycerophospholipids	RQ
PC aa C42:1	Glycerophospholipids	RQ
PC aa C42:2	Glycerophospholipids	RQ
PC aa C42:4	Glycerophospholipids	RQ
PC aa C42:5	Glycerophospholipids	RQ
PC aa C42:6	Glycerophospholipids	RQ
PC ae C30:0	Glycerophospholipids	RQ
PC ae C30:1	Glycerophospholipids	<LOD
PC ae C30:2	Glycerophospholipids	RQ
PC ae C32:1	Glycerophospholipids	RQ
PC ae C32:2	Glycerophospholipids	RQ
PC ae C34:0	Glycerophospholipids	RQ
PC ae C34:1	Glycerophospholipids	RQ
PC ae C34:2	Glycerophospholipids	RQ
PC ae C34:3	Glycerophospholipids	RQ
PC ae C36:0	Glycerophospholipids	RQ
PC ae C36:1	Glycerophospholipids	RQ
PC ae C36:2	Glycerophospholipids	RQ
PC ae C36:3	Glycerophospholipids	RQ
PC ae C36:4	Glycerophospholipids	RQ
PC ae C36:5	Glycerophospholipids	RQ
PC ae C38:0	Glycerophospholipids	RQ

Metabolite	Metabolite class	Analyte classification
PC ae C38:1	Glycerophospholipids	RQ
PC ae C38:2	Glycerophospholipids	RQ
PC ae C38:3	Glycerophospholipids	RQ
PC ae C38:4	Glycerophospholipids	RQ
PC ae C38:5	Glycerophospholipids	RQ
PC ae C38:6	Glycerophospholipids	RQ
PC ae C40:1	Glycerophospholipids	RQ
PC ae C40:2	Glycerophospholipids	RQ
PC ae C40:3	Glycerophospholipids	RQ
PC ae C40:4	Glycerophospholipids	RQ
PC ae C40:5	Glycerophospholipids	RQ
PC ae C40:6	Glycerophospholipids	RQ
PC ae C42:0	Glycerophospholipids	<LOD
PC ae C42:1	Glycerophospholipids	RQ
PC ae C42:2	Glycerophospholipids	RQ
PC ae C42:3	Glycerophospholipids	RQ
PC ae C42:4	Glycerophospholipids	RQ
PC ae C42:5	Glycerophospholipids	RQ
PC ae C44:3	Glycerophospholipids	RQ
PC ae C44:4	Glycerophospholipids	RQ
PC ae C44:5	Glycerophospholipids	RQ
PC ae C44:6	Glycerophospholipids	RQ
SM (OH) C14:1	Sphingomyelins	RQ
SM (OH) C16:1	Sphingomyelins	RQ
SM (OH) C22:1	Sphingomyelins	RQ
SM (OH) C22:2	Sphingomyelins	RQ
SM (OH) C24:1	Sphingomyelins	RQ
SM C16:0	Sphingomyelins	RQ
SM C16:1	Sphingomyelins	RQ
SM C18:0	Sphingomyelins	RQ
SM C18:1	Sphingomyelins	RQ
SM C20:2	Sphingomyelins	RQ
SM C24:0	Sphingomyelins	RQ
SM C24:1	Sphingomyelins	RQ
SM C26:0	Sphingomyelins	RQ
SM C26:1	Sphingomyelins	RQ
H1	Carbohydrates and related	Q
Cer(d16:1/18:0)	Ceramides	RQ
Cer(d16:1/20:0)	Ceramides	RQ
Cer(d16:1/22:0)	Ceramides	RQ
Cer(d16:1/23:0)	Ceramides	RQ
Cer(d16:1/24:0)	Ceramides	RQ
Cer(d18:1/14:0)	Ceramides	RQ
Cer(d18:1/16:0)	Ceramides	RQ
Cer(d18:1/18:0(OH))	Ceramides	RQ
Cer(d18:1/18:0)	Ceramides	RQ
Cer(d18:1/18:1)	Ceramides	RQ
Cer(d18:1/20:0(OH))	Ceramides	RQ
Cer(d18:1/20:0)	Ceramides	RQ
Cer(d18:1/22:0)	Ceramides	RQ
Cer(d18:1/23:0)	Ceramides	RQ
Cer(d18:1/24:0)	Ceramides	RQ
Cer(d18:1/24:1)	Ceramides	RQ
Cer(d18:1/25:0)	Ceramides	RQ
Cer(d18:1/26:0)	Ceramides	RQ
Cer(d18:1/26:1)	Ceramides	RQ

Metabolite	Metabolite class	Analyte classification
Cer(d18:2/14:0)	Ceramides	RQ
Cer(d18:2/16:0)	Ceramides	RQ
Cer(d18:2/18:0)	Ceramides	RQ
Cer(d18:2/18:1)	Ceramides	RQ
Cer(d18:2/20:0)	Ceramides	RQ
Cer(d18:2/22:0)	Ceramides	RQ
Cer(d18:2/23:0)	Ceramides	RQ
Cer(d18:2/24:0)	Ceramides	RQ
Cer(d18:2/24:1)	Ceramides	RQ
CE(14:0)	Cholesteryl esters	RQ
CE(14:1)	Cholesteryl esters	RQ
CE(15:0)	Cholesteryl esters	RQ
CE(15:1)	Cholesteryl esters	RQ
CE(16:0)	Cholesteryl esters	RQ
CE(16:1)	Cholesteryl esters	RQ
CE(17:0)	Cholesteryl esters	RQ
CE(17:1)	Cholesteryl esters	RQ
CE(18:0)	Cholesteryl esters	RQ
CE(18:1)	Cholesteryl esters	RQ
CE(18:2)	Cholesteryl esters	RQ
CE(18:3)	Cholesteryl esters	RQ
CE(20:0)	Cholesteryl esters	RQ
CE(20:1)	Cholesteryl esters	RQ
CE(20:3)	Cholesteryl esters	RQ
CE(20:4)	Cholesteryl esters	RQ
CE(20:5)	Cholesteryl esters	RQ
CE(22:0)	Cholesteryl esters	<LOD
CE(22:1)	Cholesteryl esters	RQ
CE(22:2)	Cholesteryl esters	RQ
CE(22:5)	Cholesteryl esters	RQ
CE(22:6)	Cholesteryl esters	RQ
DG(14:0_14:0)	Diacylglycerols	<LOD
DG(14:0_18:1)	Diacylglycerols	RQ
DG(14:0_18:2)	Diacylglycerols	RQ
DG(14:0_20:0)	Diacylglycerols	<LOD
DG(14:1_18:1)	Diacylglycerols	<LOD
DG(14:1_20:2)	Diacylglycerols	<LOD
DG(16:0_16:0)	Diacylglycerols	<LOD
DG(16:0_16:1)	Diacylglycerols	RQ
DG(16:0_18:1)	Diacylglycerols	RQ
DG(16:0_18:2)	Diacylglycerols	RQ
DG(16:0_20:0)	Diacylglycerols	<LOD
DG(16:0_20:3)	Diacylglycerols	<LOD
DG(16:0_20:4)	Diacylglycerols	<LOD
DG(16:1_18:0)	Diacylglycerols	RQ
DG(16:1_18:1)	Diacylglycerols	<LOD
DG(16:1_18:2)	Diacylglycerols	RQ
DG(16:1_20:0)	Diacylglycerols	<LOD
DG(17:0_17:1)	Diacylglycerols	<LOD
DG(17:0_18:1)	Diacylglycerols	RQ
DG(18:0_20:0)	Diacylglycerols	<LOD
DG(18:0_20:4)	Diacylglycerols	<LOD
DG(18:1_18:1)	Diacylglycerols	RQ
DG(18:1_18:2)	Diacylglycerols	RQ
DG(18:1_18:3)	Diacylglycerols	<LOD
DG(18:1_18:4)	Diacylglycerols	<LOD

Metabolite	Metabolite class	Analyte classification
DG(18:1_20:0)	Diacylglycerols	RQ
DG(18:1_20:1)	Diacylglycerols	RQ
DG(18:1_20:2)	Diacylglycerols	RQ
DG(18:1_20:3)	Diacylglycerols	RQ
DG(18:1_20:4)	Diacylglycerols	<LOD
DG(18:1_22:5)	Diacylglycerols	<LOD
DG(18:1_22:6)	Diacylglycerols	<LOD
DG(18:2_18:2)	Diacylglycerols	RQ
DG(18:2_18:3)	Diacylglycerols	<LOD
DG(18:2_18:4)	Diacylglycerols	<LOD
DG(18:2_20:0)	Diacylglycerols	RQ
DG(18:2_20:4)	Diacylglycerols	<LOD
DG(18:3_18:3)	Diacylglycerols	<LOD
DG(18:3_20:2)	Diacylglycerols	<LOD
DG(21:0_22:6)	Diacylglycerols	<LOD
DG(22:1_22:2)	Diacylglycerols	<LOD
DG-O(14:0_18:2)	Diacylglycerols	<LOD
DG-O(16:0_18:1)	Diacylglycerols	<LOD
DG-O(18:2_18:2)	Diacylglycerols	RQ
Cer(d18:0/18:0(OH))	Dihydroceramides	RQ
Cer(d18:0/18:0)	Dihydroceramides	RQ
Cer(d18:0/20:0)	Dihydroceramides	<LOD
Cer(d18:0/22:0)	Dihydroceramides	RQ
Cer(d18:0/24:0)	Dihydroceramides	RQ
Cer(d18:0/24:1)	Dihydroceramides	RQ
Cer(d18:0/26:1(OH))	Dihydroceramides	<LOD
Cer(d18:0/26:1)	Dihydroceramides	<LOD
Hex2Cer(d18:1/14:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/16:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/18:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/20:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/22:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/24:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/24:1)	Glycosylceramides	RQ
Hex2Cer(d18:1/26:0)	Glycosylceramides	RQ
Hex2Cer(d18:1/26:1)	Glycosylceramides	RQ
Hex3Cer(d18:1/16:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/18:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/20:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/22:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/24:1)	Glycosylceramides	RQ
Hex3Cer(d18:1/26:1)	Glycosylceramides	RQ
HexCer(d16:1/20:0)	Glycosylceramides	RQ
HexCer(d16:1/22:0)	Glycosylceramides	RQ
HexCer(d16:1/24:0)	Glycosylceramides	RQ
HexCer(d18:1/14:0)	Glycosylceramides	RQ
HexCer(d18:1/16:0)	Glycosylceramides	RQ
HexCer(d18:1/18:0)	Glycosylceramides	RQ
HexCer(d18:1/18:1)	Glycosylceramides	RQ
HexCer(d18:1/20:0)	Glycosylceramides	RQ
HexCer(d18:1/22:0)	Glycosylceramides	RQ
HexCer(d18:1/23:0)	Glycosylceramides	RQ
HexCer(d18:1/24:0)	Glycosylceramides	RQ
HexCer(d18:1/24:1)	Glycosylceramides	RQ
HexCer(d18:1/26:0)	Glycosylceramides	RQ
HexCer(d18:1/26:1)	Glycosylceramides	RQ

Metabolite	Metabolite class	Analyte classification
HexCer(d18:2/16:0)	Glycosylceramides	RQ
HexCer(d18:2/18:0)	Glycosylceramides	RQ
HexCer(d18:2/20:0)	Glycosylceramides	RQ
HexCer(d18:2/22:0)	Glycosylceramides	RQ
HexCer(d18:2/23:0)	Glycosylceramides	RQ
HexCer(d18:2/24:0)	Glycosylceramides	RQ
TG(14:0_32:2)	Triacylglycerols	RQ
TG(14:0_34:0)	Triacylglycerols	RQ
TG(14:0_34:1)	Triacylglycerols	RQ
TG(14:0_34:2)	Triacylglycerols	RQ
TG(14:0_34:3)	Triacylglycerols	RQ
TG(14:0_35:1)	Triacylglycerols	RQ
TG(14:0_35:2)	Triacylglycerols	RQ
TG(14:0_36:1)	Triacylglycerols	RQ
TG(14:0_36:2)	Triacylglycerols	RQ
TG(14:0_36:3)	Triacylglycerols	RQ
TG(14:0_36:4)	Triacylglycerols	RQ
TG(14:0_38:4)	Triacylglycerols	RQ
TG(14:0_38:5)	Triacylglycerols	RQ
TG(14:0_40:5)	Triacylglycerols	RQ
TG(16:0_28:1)	Triacylglycerols	RQ
TG(16:0_28:2)	Triacylglycerols	RQ
TG(16:0_30:2)	Triacylglycerols	RQ
TG(16:0_32:0)	Triacylglycerols	RQ
TG(16:0_32:1)	Triacylglycerols	RQ
TG(16:0_32:2)	Triacylglycerols	RQ
TG(16:0_32:3)	Triacylglycerols	RQ
TG(16:0_33:1)	Triacylglycerols	RQ
TG(16:0_33:2)	Triacylglycerols	RQ
TG(16:0_34:0)	Triacylglycerols	RQ
TG(16:0_34:1)	Triacylglycerols	RQ
TG(16:0_34:2)	Triacylglycerols	RQ
TG(16:0_34:3)	Triacylglycerols	RQ
TG(16:0_34:4)	Triacylglycerols	RQ
TG(16:0_35:1)	Triacylglycerols	RQ
TG(16:0_35:2)	Triacylglycerols	RQ
TG(16:0_35:3)	Triacylglycerols	RQ
TG(16:0_36:2)	Triacylglycerols	RQ
TG(16:0_36:3)	Triacylglycerols	RQ
TG(16:0_36:4)	Triacylglycerols	RQ
TG(16:0_36:5)	Triacylglycerols	RQ
TG(16:0_36:6)	Triacylglycerols	RQ
TG(16:0_37:3)	Triacylglycerols	RQ
TG(16:0_38:1)	Triacylglycerols	RQ
TG(16:0_38:2)	Triacylglycerols	RQ
TG(16:0_38:3)	Triacylglycerols	RQ
TG(16:0_38:4)	Triacylglycerols	RQ
TG(16:0_38:5)	Triacylglycerols	RQ
TG(16:0_38:6)	Triacylglycerols	RQ
TG(16:0_38:7)	Triacylglycerols	RQ
TG(16:0_40:6)	Triacylglycerols	RQ
TG(16:0_40:7)	Triacylglycerols	RQ
TG(16:0_40:8)	Triacylglycerols	RQ
TG(16:1_28:0)	Triacylglycerols	RQ
TG(16:1_30:1)	Triacylglycerols	RQ
TG(16:1_32:0)	Triacylglycerols	RQ

Metabolite	Metabolite class	Analyte classification
TG(16:1_32:1)	Triacylglycerols	RQ
TG(16:1_32:2)	Triacylglycerols	RQ
TG(16:1_33:1)	Triacylglycerols	RQ
TG(16:1_34:0)	Triacylglycerols	RQ
TG(16:1_34:1)	Triacylglycerols	RQ
TG(16:1_34:2)	Triacylglycerols	RQ
TG(16:1_34:3)	Triacylglycerols	RQ
TG(16:1_36:1)	Triacylglycerols	RQ
TG(16:1_36:2)	Triacylglycerols	RQ
TG(16:1_36:3)	Triacylglycerols	RQ
TG(16:1_36:4)	Triacylglycerols	RQ
TG(16:1_36:5)	Triacylglycerols	RQ
TG(16:1_38:3)	Triacylglycerols	RQ
TG(16:1_38:4)	Triacylglycerols	RQ
TG(16:1_38:5)	Triacylglycerols	RQ
TG(17:0_32:1)	Triacylglycerols	RQ
TG(17:0_34:1)	Triacylglycerols	RQ
TG(17:0_34:2)	Triacylglycerols	RQ
TG(17:0_34:3)	Triacylglycerols	RQ
TG(17:0_36:3)	Triacylglycerols	RQ
TG(17:0_36:4)	Triacylglycerols	RQ
TG(17:1_32:1)	Triacylglycerols	RQ
TG(17:1_34:1)	Triacylglycerols	RQ
TG(17:1_34:2)	Triacylglycerols	RQ
TG(17:1_34:3)	Triacylglycerols	RQ
TG(17:1_36:3)	Triacylglycerols	RQ
TG(17:1_36:4)	Triacylglycerols	RQ
TG(17:1_36:5)	Triacylglycerols	RQ
TG(17:1_38:5)	Triacylglycerols	RQ
TG(17:1_38:6)	Triacylglycerols	RQ
TG(17:1_38:7)	Triacylglycerols	RQ
TG(17:2_34:2)	Triacylglycerols	RQ
TG(17:2_34:3)	Triacylglycerols	RQ
TG(17:2_36:2)	Triacylglycerols	RQ
TG(17:2_36:3)	Triacylglycerols	RQ
TG(17:2_36:4)	Triacylglycerols	RQ
TG(17:2_38:5)	Triacylglycerols	RQ
TG(17:2_38:6)	Triacylglycerols	RQ
TG(17:2_38:7)	Triacylglycerols	RQ
TG(18:0_30:0)	Triacylglycerols	RQ
TG(18:0_30:1)	Triacylglycerols	RQ
TG(18:0_32:0)	Triacylglycerols	RQ
TG(18:0_32:1)	Triacylglycerols	RQ
TG(18:0_32:2)	Triacylglycerols	RQ
TG(18:0_34:2)	Triacylglycerols	RQ
TG(18:0_34:3)	Triacylglycerols	RQ
TG(18:0_36:1)	Triacylglycerols	RQ
TG(18:0_36:2)	Triacylglycerols	RQ
TG(18:0_36:3)	Triacylglycerols	RQ
TG(18:0_36:4)	Triacylglycerols	RQ
TG(18:0_36:5)	Triacylglycerols	RQ
TG(18:0_38:6)	Triacylglycerols	RQ
TG(18:0_38:7)	Triacylglycerols	RQ
TG(18:1_26:0)	Triacylglycerols	RQ
TG(18:1_28:1)	Triacylglycerols	RQ

Metabolite	Metabolite class	Analyte classification
TG(18:1_30:0)	Triacylglycerols	RQ
TG(18:1_30:1)	Triacylglycerols	RQ
TG(18:1_30:2)	Triacylglycerols	RQ
TG(18:1_31:0)	Triacylglycerols	RQ
TG(18:1_32:0)	Triacylglycerols	RQ
TG(18:1_32:1)	Triacylglycerols	RQ
TG(18:1_32:2)	Triacylglycerols	RQ
TG(18:1_32:3)	Triacylglycerols	RQ
TG(18:1_33:0)	Triacylglycerols	RQ
TG(18:1_33:1)	Triacylglycerols	RQ
TG(18:1_33:2)	Triacylglycerols	RQ
TG(18:1_33:3)	Triacylglycerols	RQ
TG(18:1_34:1)	Triacylglycerols	RQ
TG(18:1_34:2)	Triacylglycerols	RQ
TG(18:1_34:3)	Triacylglycerols	RQ
TG(18:1_34:4)	Triacylglycerols	RQ
TG(18:1_35:2)	Triacylglycerols	RQ
TG(18:1_35:3)	Triacylglycerols	RQ
TG(18:1_36:0)	Triacylglycerols	RQ
TG(18:1_36:1)	Triacylglycerols	RQ
TG(18:1_36:2)	Triacylglycerols	RQ
TG(18:1_36:3)	Triacylglycerols	RQ
TG(18:1_36:4)	Triacylglycerols	RQ
TG(18:1_36:5)	Triacylglycerols	RQ
TG(18:1_36:6)	Triacylglycerols	RQ
TG(18:1_38:5)	Triacylglycerols	RQ
TG(18:1_38:6)	Triacylglycerols	RQ
TG(18:1_38:7)	Triacylglycerols	RQ
TG(18:2_28:0)	Triacylglycerols	RQ
TG(18:2_30:0)	Triacylglycerols	RQ
TG(18:2_30:1)	Triacylglycerols	RQ
TG(18:2_31:0)	Triacylglycerols	RQ
TG(18:2_32:0)	Triacylglycerols	RQ
TG(18:2_32:1)	Triacylglycerols	RQ
TG(18:2_32:2)	Triacylglycerols	RQ
TG(18:2_33:0)	Triacylglycerols	RQ
TG(18:2_33:1)	Triacylglycerols	RQ
TG(18:2_33:2)	Triacylglycerols	RQ
TG(18:2_34:0)	Triacylglycerols	RQ
TG(18:2_34:1)	Triacylglycerols	RQ
TG(18:2_34:2)	Triacylglycerols	RQ
TG(18:2_34:3)	Triacylglycerols	RQ
TG(18:2_34:4)	Triacylglycerols	RQ
TG(18:2_35:1)	Triacylglycerols	RQ
TG(18:2_35:2)	Triacylglycerols	RQ
TG(18:2_35:3)	Triacylglycerols	RQ
TG(18:2_36:0)	Triacylglycerols	RQ
TG(18:2_36:1)	Triacylglycerols	RQ
TG(18:2_36:2)	Triacylglycerols	RQ
TG(18:2_36:3)	Triacylglycerols	RQ
TG(18:2_36:4)	Triacylglycerols	RQ
TG(18:2_36:5)	Triacylglycerols	RQ
TG(18:2_38:4)	Triacylglycerols	RQ
TG(18:2_38:5)	Triacylglycerols	RQ
TG(18:2_38:6)	Triacylglycerols	RQ

Metabolite	Metabolite class	Analyte classification
TG(18:3_30:0)	Triacylglycerols	RQ
TG(18:3_32:0)	Triacylglycerols	RQ
TG(18:3_32:1)	Triacylglycerols	RQ
TG(18:3_33:2)	Triacylglycerols	RQ
TG(18:3_34:0)	Triacylglycerols	RQ
TG(18:3_34:1)	Triacylglycerols	RQ
TG(18:3_34:2)	Triacylglycerols	RQ
TG(18:3_34:3)	Triacylglycerols	RQ
TG(18:3_35:2)	Triacylglycerols	RQ
TG(18:3_36:1)	Triacylglycerols	RQ
TG(18:3_36:2)	Triacylglycerols	RQ
TG(18:3_36:3)	Triacylglycerols	RQ
TG(18:3_36:4)	Triacylglycerols	RQ
TG(18:3_38:5)	Triacylglycerols	RQ
TG(18:3_38:6)	Triacylglycerols	RQ
TG(20:0_32:3)	Triacylglycerols	RQ
TG(20:0_32:4)	Triacylglycerols	RQ
TG(20:0_34:1)	Triacylglycerols	RQ
TG(20:1_24:3)	Triacylglycerols	<LOD
TG(20:1_26:1)	Triacylglycerols	<LOD
TG(20:1_30:1)	Triacylglycerols	RQ
TG(20:1_32:0)	Triacylglycerols	RQ
TG(20:1_32:1)	Triacylglycerols	RQ
TG(20:1_32:2)	Triacylglycerols	RQ
TG(20:1_32:3)	Triacylglycerols	RQ
TG(20:1_34:0)	Triacylglycerols	RQ
TG(20:1_34:1)	Triacylglycerols	RQ
TG(20:1_34:2)	Triacylglycerols	RQ
TG(20:1_34:3)	Triacylglycerols	RQ
TG(20:2_32:0)	Triacylglycerols	RQ
TG(20:2_32:1)	Triacylglycerols	RQ
TG(20:2_34:1)	Triacylglycerols	RQ
TG(20:2_34:2)	Triacylglycerols	RQ
TG(20:2_34:3)	Triacylglycerols	RQ
TG(20:2_34:4)	Triacylglycerols	RQ
TG(20:2_36:5)	Triacylglycerols	RQ
TG(20:3_32:0)	Triacylglycerols	RQ
TG(20:3_32:1)	Triacylglycerols	RQ
TG(20:3_32:2)	Triacylglycerols	RQ
TG(20:3_34:0)	Triacylglycerols	RQ
TG(20:3_34:1)	Triacylglycerols	RQ
TG(20:3_34:2)	Triacylglycerols	RQ
TG(20:3_34:3)	Triacylglycerols	RQ
TG(20:3_36:3)	Triacylglycerols	RQ
TG(20:3_36:4)	Triacylglycerols	RQ
TG(20:3_36:5)	Triacylglycerols	RQ
TG(20:4_30:0)	Triacylglycerols	RQ
TG(20:4_32:0)	Triacylglycerols	RQ
TG(20:4_32:1)	Triacylglycerols	RQ
TG(20:4_32:2)	Triacylglycerols	RQ
TG(20:4_33:2)	Triacylglycerols	RQ
TG(20:4_34:0)	Triacylglycerols	RQ
TG(20:4_34:1)	Triacylglycerols	RQ
TG(20:4_34:2)	Triacylglycerols	RQ
TG(20:4_34:3)	Triacylglycerols	RQ
TG(20:4_35:3)	Triacylglycerols	RQ
TG(20:4_36:2)	Triacylglycerols	RQ
TG(20:4_36:3)	Triacylglycerols	RQ
TG(20:4_36:4)	Triacylglycerols	RQ
TG(20:4_36:5)	Triacylglycerols	RQ
TG(20:5_34:0)	Triacylglycerols	RQ
TG(20:5_34:1)	Triacylglycerols	RQ
TG(20:5_34:2)	Triacylglycerols	RQ
TG(20:5_36:2)	Triacylglycerols	RQ
TG(20:5_36:3)	Triacylglycerols	RQ
TG(22:0_32:4)	Triacylglycerols	<LOD
TG(22:1_32:5)	Triacylglycerols	<LOD
TG(22:2_32:4)	Triacylglycerols	<LOD
TG(22:3_30:2)	Triacylglycerols	<LOD
TG(22:4_32:0)	Triacylglycerols	RQ
TG(22:4_32:2)	Triacylglycerols	RQ
TG(22:4_34:2)	Triacylglycerols	RQ
TG(22:5_32:0)	Triacylglycerols	RQ
TG(22:5_32:1)	Triacylglycerols	RQ
TG(22:5_34:1)	Triacylglycerols	RQ
TG(22:5_34:2)	Triacylglycerols	RQ
TG(22:5_34:3)	Triacylglycerols	RQ
TG(22:6_32:0)	Triacylglycerols	RQ
TG(22:6_32:1)	Triacylglycerols	RQ
TG(22:6_34:1)	Triacylglycerols	RQ
TG(22:6_34:2)	Triacylglycerols	RQ
TG(22:6_34:3)	Triacylglycerols	RQ

Analyte classes and classifications of analytical performance with FIA-MS/MS.

Abbreviations: LOD, limit of detection; Q, quantitative; QR, quantitative with restrictions; and RQ, relative quantitative.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-XS Triple Quadrupole Mass Spectrometry <https://www.waters.com/134889751>

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

720006827, April 2020