

Standardized Targeted Metabolomics Using the BIOCRATES MxP® Quant 500 Kit on the SCIEX Triple Quad™ 5500+ Mass Spectrometer

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1 Introduction

Metabolic signatures can provide crucial insights into the 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 SCIEX Triple Quad™ 5500+ LC-MS/MS system. The assay offers multiplexed MS/MS analysis of up to 630 metabolites and lipids from 26 analyte classes in only 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 BIOCRATES' Met/DQ™ 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, 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).

The SCIEX Triple Quad 5500+ mass spectrometer offers a broader dynamic ranged and increased sensitivity compared to its predecessors. Analytical validation was performed on this platform coupled with an Agilent 1290 Infinity II UHPLC frontend for human plasma, following EMA and FDA guidelines.

LC-MS/MS (13 small molecule classes)	FIA-MS/MS (hexoses & 12 lipid 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)	- 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.

2 Materials and Method

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 user manual. Blank and zero samples, 7 calibration standards, 3 levels of quality control samples (human plasma-based QCs) and a variety of plasma samples were subjected to two UHPLC-ESI-MS/MS analyses in multiple reaction monitoring (MRM) mode, followed by two FIA-MS/MS runs. Figure 2 shows two representative

chromatograms (positive & negative ion modes) for a human plasma sample.

For quantitation, both LC and FIA data were converted and imported directly into the BIOCRATES software Met/DQ™ Oxygen and quantified. Met/DQ™ includes an automated 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.

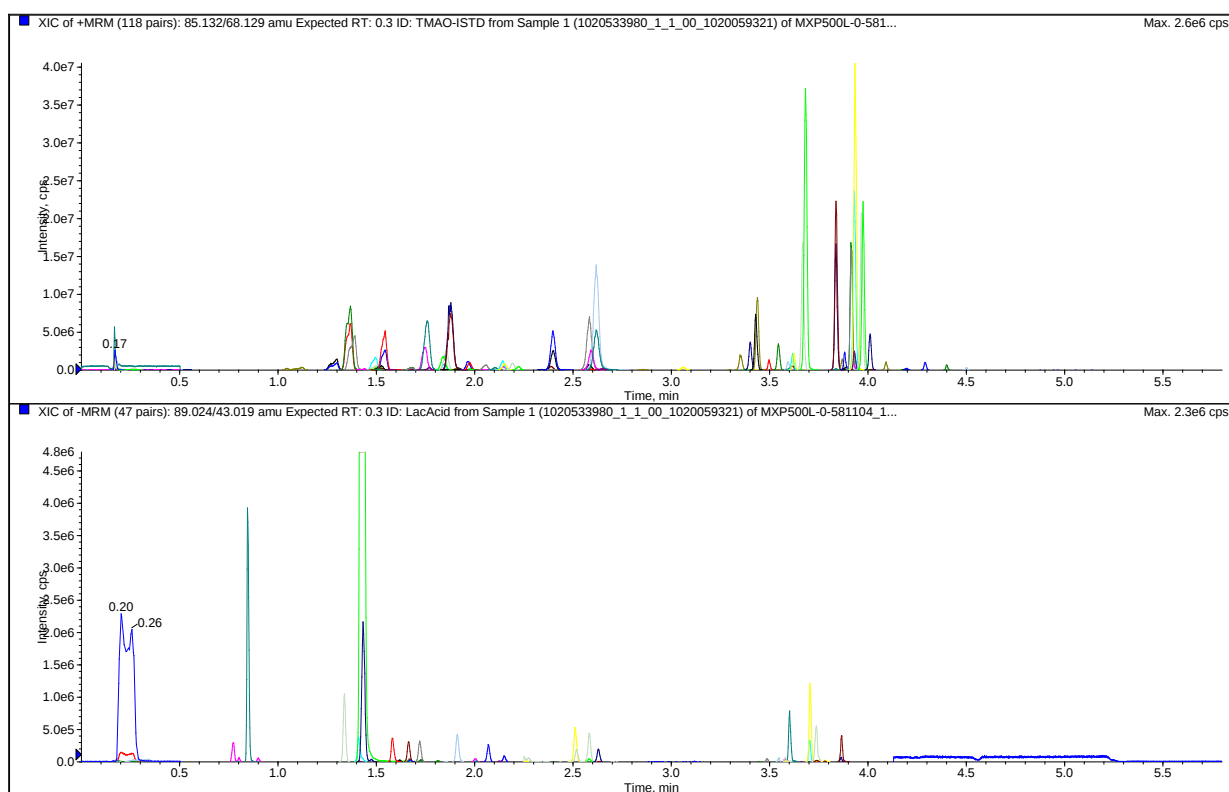


Figure 2: LC extracted ion chromatograms (XIC) derived from a human plasma sample in positive (upper panel) and negative (lower panel) ion modes, measured with the BIOCRATES MxP® Quant 500 kit on the Agilent 1290 infinity II UHPLC – SCIEX Triple Quad™ 5500+ system.

3 Results and Discussion

The adaption of the ready-to-use MxP® Quant 500 kit to the Triple Quad™ 5500+ LC-MS/MS system comprised the optimization of instrumental parameters from sample preparation setup to mass spectrometric features.

Overall, analysis times were 6 min per LC-MS/MS run and 3 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 less than 42 hours.

Validation: Summary

A comprehensive set of defined human plasma samples was used for the validation of the MxP® Quant 500 kit on SCIEX Triple Quad™ 5500+ mass spectrometer coupled with an Agilent 1290 Infinity II UHPLC. Table 2 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 7-point calibrated analytes and their calibrants above the limit of detection (LOD) in the course of the validation, therefor classified as “quantitative”.

LC analytes which 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 SCIEX Triple Quad™ 5500+ system. Excellent accuracy between 85-115% were 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.

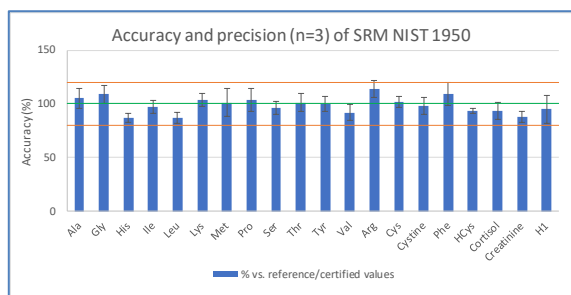


Figure 3: Accuracy and precision of measurements compared to reference and/or certified NIST SRM 1950 values (n=3).

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 Table 3. 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 490-500 small molecules and lipids could be routinely analyzed (>LOD) and quantified with the MxP® Quant 500 kit. The kit is, therefore, highly suited for comparative studies of plasma from different sample cohorts.

In addition, the MxP® Quant 500 kit on the SCIEX Triple Quad™ 5500+ mass spectrometer was also evaluated for rat plasma, mouse liver homogenate, and human fecal samples. Table 1 shows the representative number of analytes that were quantified in these matrices. The fecal sample analysis is particularly relevant for microbiome research. 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.

The analytical performance of the MxP® Quant 500 kit on the SCIEX Triple Quad™ 5500+ mass spectrometer was also compared to its predecessor, the SCIEX QTRAP® 5500 instrument. The former has a broader dynamic range of detection than the latter, which result in a wider concentration range coverage of the target metabolites. Overall, the performance of the MxP® Quant 500 kit on the SCIEX Triple Quad™ 5500+ is very similar to that achieved on the SCIEX QTRAP® 5500.

4 Conclusions

The data shown in this application note supports the high reliability and analytical potency of the MxP® Quant 500 kit on the SCIEX Triple Quad™ 5500+ system, delivering high level of global standardization in metabolomics with excellent lab-to-lab comparability.

Table 1: Number of detectable (> LOD, grey) and quantifiable (> LOD with CV < 20%, in brackets) analytes in each class, using the MxP® Quant 500 kit on the SCIEX Triple Quad™ 5500+ for the analysis of different sample matrices.

Analyte Class	Human Plasma	Human Feces	Rat Plasma	Mouse Liver
LC-MS/MS				
Alkaloids	1 (1)	1 (1)	1 (1)	1 (1)
Amine oxides	1 (1)	0 (0)	1 (1)	1 (1)
Amino acids	20 (20)	19 (18)	20 (20)	19 (19)
Amino acid related	22 (20)	26 (22)	24 (24)	21 (20)
Bile acids	14 (14)	14 (13)	14 (14)	12 (11)
Biogenic amines	4 (4)	8 (8)	6 (6)	6 (6)
Carboxylic acids	3 (3)	4 (3)	7 (7)	6 (6)
Cresols	1 (1)	1 (1)	1 (1)	1 (1)
Fatty acids	7 (7)	8 (8)	7 (7)	8 (8)
Hormones and related	2 (2)	2 (1)	0 (0)	1 (1)
Indoles and derivatives	3 (3)	4 (4)	3 (3)	2 (2)
Nucleobases and related	2 (2)	2 (2)	0 (0)	2 (2)
Vitamins and cofactors	1 (1)	1 (1)	1 (1)	1 (1)
<i>Sub-total LC-MS/MS</i>	<i>81 (79)</i>	<i>90 (82)</i>	<i>85 (85)</i>	<i>81 (79)</i>
FIA-MS/MS				
Acylcarnitines	15 (14)	9 (8)	15 (13)	20 (19)
Ceramides	26 (20)	23 (15)	21 (14)	22 (18)
Cholesteryl esters	21 (21)	1 (0)	20 (18)	1 (0)
Diacylglycerols	20 (17)	14 (14)	11 (10)	25 (24)
Dihydroceramides	4 (2)	2 (2)	0 (0)	0 (0)
Lysophosphatidylcholines	12 (12)	6 (6)	12 (12)	11 (11)
Phosphatidylcholines	70 (68)	33 (29)	69 (68)	69 (69)
Dihexosylceramides	8 (7)	4 (3)	6 (4)	3 (1)
Trihexosylceramides	5 (2)	0 (0)	2 (1)	1 (1)
Hexosylceramides	17 (13)	5 (5)	12 (12)	8 (8)
Sphingomyelins	14 (14)	4 (4)	15 (12)	12 (11)
Carbohydrates and related	1 (1)	1 (1)	1 (1)	1 (1)
Triacylglycerols	237 (221)	11 (7)	220 (200)	5 (0)
<i>Sub-total FIA-MS/MS</i>	<i>450 (412)</i>	<i>113 (94)</i>	<i>404 (365)</i>	<i>178 (163)</i>
Total	531 (491)	203 (176)	489 (450)	259 (242)

Table 2: Analyte classes & classification of analytical performance with LC-MS/MS

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

Analyte	Class	Analytical Classification
Trigonelline	Alkaloids	QR
TMAO	Amine oxides	QR
Ala	Amino acids	Q
Arg	Amino acids	Q
Asn	Amino acids	Q
Asp	Amino acids	QR
Cys	Amino acids	RQ
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 acids related	QR
3-Met-His	Amino acids related	QR
5-AVA	Amino acids related	QR
AABA	Amino acids related	QR
ADMA	Amino acids related	Q
Ac-Orn	Amino acids related	Q
Anserine	Amino acids related	QR
BABA	Amino acids related	QR
Betaine	Amino acids related	QR
Carnosine	Amino acids related	Q
Cit	Amino acids related	Q
Creatinine	Amino acids related	Q
Cystine	Amino acids related	RQ
DOPA	Amino acids related	Q
HArg	Amino acids related	QR
HCys	Amino acids related	RQ
Kynurenine	Amino acids related	Q
Met-SO	Amino acids related	Q
Nitro-Tyr	Amino acids related	QR
Orn	Amino acids related	QR
PAG	Amino acids related	QR
PheAlaBetaine	Amino acids related	QR
ProBetaine	Amino acids related	QR
SDMA	Amino acids related	Q
Sarcosine	Amino acids related	Q
Taurine	Amino acids related	Q
TrpBetaine	Amino acids related	QR
alpha-AAA	Amino acids related	Q
c4-OH-Pro	Amino acids related	Q
t4-OH-Pro	Amino acids related	Q
CA	Bile acids	QR

Analyte	Class	Analytical 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
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
beta-Ala	Biogenic amines	QR
AconAcid	Carboxylic acids	RQ
DiCA(12:0)	Carboxylic acids	RQ
DiCA(14:0)	Carboxylic acids	RQ
HipAcid	Carboxylic acids	QR
Lac	Carboxylic acids	QR
OH-GlutAcid	Carboxylic acids	RQ
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	<LOD
FA(14:0)	Fatty acids	<LOD
FA(16:0)	Fatty acids	<LOD
FA(18:0)	Fatty acids	<LOD
FA(18:1)	Fatty acids	RQ
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	RQ
Hypoxanthine	Nucleobases and related	QR
Xanthine	Nucleobases and related	QR
Choline	Vitamins and cofactors	QR

Table 3: Analyte classes & classifications of analytical performance with FIA-MS/MS

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

Analyte	Class	Analytical Classification
C0	Acylcarnitines	Q
C10	Acylcarnitines	Q
C10:1	Acylcarnitines	<LOD
C10:2	Acylcarnitines	<LOD
C12	Acylcarnitines	Q
C12:1	Acylcarnitines	<LOD
C12-DC	Acylcarnitines	<LOD
C14	Acylcarnitines	Q
C14:1	Acylcarnitines	RQ
C14:1-OH	Acylcarnitines	RQ
C14:2	Acylcarnitines	RQ
C14:2-OH	Acylcarnitines	RQ
C16	Acylcarnitines	Q
C16:1	Acylcarnitines	<LOD
C16:1-OH	Acylcarnitines	<LOD
C16:2	Acylcarnitines	RQ
C16:2-OH	Acylcarnitines	<LOD
C16-OH	Acylcarnitines	<LOD
C18	Acylcarnitines	Q
C18:1	Acylcarnitines	RQ
C18:1-OH	Acylcarnitines	<LOD
C18:2	Acylcarnitines	RQ
C2	Acylcarnitines	Q
C3	Acylcarnitines	Q
C3:1	Acylcarnitines	<LOD
C3-DC (C4-OH)	Acylcarnitines	RQ
C3-OH	Acylcarnitines	<LOD
C4	Acylcarnitines	Q
C4:1	Acylcarnitines	< LOD
C5	Acylcarnitines	Q
C5:1	Acylcarnitines	< LOD
C5:1-DC	Acylcarnitines	< LOD
C5-DC (C6-OH)	Acylcarnitines	< LOD
C5-M-DC	Acylcarnitines	< LOD
C5-OH (C3-DC-M)	Acylcarnitines	< LOD
C6 (C4:1-DC)	Acylcarnitines	Q
C6:1	Acylcarnitines	<LOD

Analyte	Class	Analytical Classification
C7-DC	Acylcarnitines	<LOD
C8	Acylcarnitines	Q
C9	Acylcarnitines	RQ
Cer(d16:1/18:0)	Ceramides	<LOD
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	<LOD
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	<LOD
Cer(d18:1/20:0(OH))	Ceramides	<LOD
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	<LOD
Cer(d18:2/14:0)	Ceramides	<LOD
Cer(d18:2/16:0)	Ceramides	RQ
Cer(d18:2/18:0)	Ceramides	<LOD
Cer(d18:2/18:1)	Ceramides	<LOD
Cer(d18:2/20:0)	Ceramides	<LOD
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

Analyte	Class	Analytical Classification
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	RQ
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	<LOD
DG(14:0_18:2)	Diacylglycerols	<LOD
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	<LOD
DG(16:1_18:1)	Diacylglycerols	RQ
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	<LOD
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

Analyte	Class	Analytical Classification
DG(18:1_18:3)	Diacylglycerols	RQ
DG(18:1_18:4)	Diacylglycerols	<LOD
DG(18:1_20:0)	Diacylglycerols	<LOD
DG(18:1_20:1)	Diacylglycerols	RQ
DG(18:1_20:2)	Diacylglycerols	<LOD
DG(18:1_20:3)	Diacylglycerols	<LOD
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	RQ
DG(18:2_18:4)	Diacylglycerols	<LOD
DG(18:2_20:0)	Diacylglycerols	<LOD
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(16:0_20:4)	Diacylglycerols	<LOD
Cer(d18:0/18:0(OH))	Dihydroceramides	RQ
Cer(d18:0/18:0)	Dihydroceramides	<LOD
Cer(d18:0/20:0)	Dihydroceramides	<LOD
Cer(d18:0/22:0)	Dihydroceramides	<LOD
Cer(d18:0/24:0)	Dihydroceramides	RQ
Cer(d18:0/24:1)	Dihydroceramides	<LOD
Cer(d18:0/26:1(OH))	Dihydroceramides	<LOD
Cer(d18:0/26:1)	Dihydroceramides	<LOD
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

Analyte	Class	Analytical Classification
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 C30:2	Glycerophospholipids	<LOD
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 C38:1	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	<LOD
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

Analyte	Class	Analytical Classification
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
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

Analyte	Class	Analytical Classification
PC ae C44:5	Glycerophospholipids	RQ
PC ae C44:6	Glycerophospholipids	RQ
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	<LOD
Hex2Cer(d18:1/26:1)	Glycosylceramides	<LOD
Hex3Cer(d18:1/16:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/18:0)	Glycosylceramides	RQ
Hex3Cer(d18:1/24:1)	Glycosylceramides	RQ
Hex3Cer(d18:1/26:1)	Glycosylceramides	RQ
Hex3Cer(d18:1_20:0)	Glycosylceramides	RQ
Hex3Cer(d18:1_22:0)	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	<LOD
HexCer(d18:1/26:1)	Glycosylceramides	<LOD
HexCer(d18:2/16:0)	Glycosylceramides	<LOD
HexCer(d18:2/18:0)	Glycosylceramides	<LOD
HexCer(d18:2/20:0)	Glycosylceramides	<LOD
HexCer(d18:2/22:0)	Glycosylceramides	RQ
HexCer(d18:2/23:0)	Glycosylceramides	RQ
HexCer(d18:2/24:0)	Glycosylceramides	RQ
SM (OH) C14:1	Sphingomyelins	RQ
SM (OH) C16:1	Sphingomyelins	RQ
SM (OH) C22:1	Sphingomyelins	RQ

Analyte	Class	Analytical Classification
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 C22:3	Sphingomyelins	<LOD
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
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_39:3)	Triacylglycerols	<LOD
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

Analyte	Class	Analytical Classification
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
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

Analyte	Class	Analytical Classification
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	<LOD
TG(17:1_38:7)	Triacylglycerols	<LOD
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	<LOD
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

Analyte	Class	Analytical Classification
TG(18:1_28:1)	Triacylglycerols	RQ
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

Analyte	Class	Analytical Classification
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
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	<LOD
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_31:0)	Triacylglycerols	<LOD

Analyte	Class	Analytical Classification
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	<LOD
TG(20:2_36:5)	Triacylglycerols	<LOD
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

Analyte	Class	Analytical Classification
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