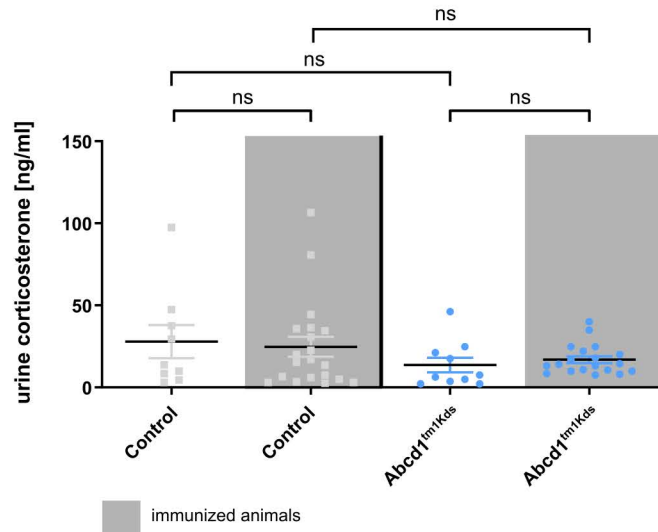
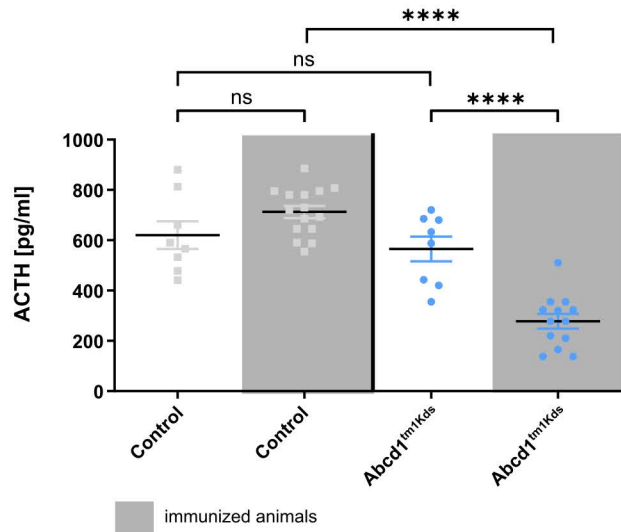


(A)**(B)**

AbsoluteIDQ[®] kit

Analytical specifications AbsoluteIDQ[®] p180
biocrates.com



Table of contents

1	General remarks	4
1.1	Produce! notes.....	4
1.2	Limitations of the AbsoluteIDQ® p180 kit	5
1.3	Mass spectrometers.....	6
1.4	Calibration standards	7
1.5	Quality control.....	8
2	Analytical performance characteristics.....	9
2.1	Overview	9
2.2	Specificity	19
2.3	Calibration, precision and accuracy.....	20
2.3.1	LC-MS/MS part of the AbsoluteIDQ® p180 kit.....	20
2.3.2	FIA-MS/MS Part of the AbsoluteIDQ® p180 kit.....	26
2.4	Functional sensitivity	28
2.5	Reproducibility and accuracy	28
2.6	Stability.....	31
	Ordering and technical support	32
	Sales	32
	Technical assistance	32

The information in these analytical specifications document is subject to change without notice and should not be construed as a commitment by biocrates life sciences ag to assume responsibility for any errors that may appear. The AbsoluteIDQ® p180 kit is for research use only and not for use in diagnostic procedures.

While every precaution has been taken in the preparation of this analytical specifications, biocrates® life sciences ag shall not be liable for punitive, incidental, or consequential damage in connection with or arising from the use of this document.

AbsoluteIDQ® and MetIDQ™ are trademarks of biocrates® life sciences ag. All other trademarks are the sole property of their respective owners. It is not intended to encourage use of these products in any manner that might infringe on the intellectual property rights of others.

Copyright © biocrates life sciences ag 2020. All rights reserved.

biocrates® is a registered trademark of biocrates® life sciences ag. Procedures, methods and components of the AbsoluteIDQ® kit and the MetIDQ™ software are patent pending. For brevity, MetIDQ may be used in place of the full product name MetIDQ™ and kit may be used in place of the full product name AbsoluteIDQ® p180 kit in descriptions and tables in this document.

For research only | not for use in diagnostic procedures.



biocrates life sciences ag

Eduard-Bodem-Gasse 8

6020 Innsbruck

Austria

Phone: +43 512 579 823

Fax: +43 512 579 823 329

office@biocrates.com

www.biocrates.com

Document version: 6

Date of the last 2020-11-09

Filename: AS-p180 (v1-2020)

1 General remarks

The AbsoluteIDQ® p180 kit was validated with human EDTA plasma. Both, EDTA and heparin are suitable anticoagulants. Due to its biological similarity, human serum can also be used without impairing the Analytical performance. For other biological matrices and species, please refer to our application notes that you find on the USB stick and on our homepage. Here you also find guidelines for collecting blood and tissue samples ("Guidelines sample preparation.pdf")

The analytical principle of the AbsoluteIDQ® p180 kit is based on a complex combination of several experimental steps. This makes it necessary to follow the user manual in detail and gives rise to method-dependent limitations of the analysis that are described overleaf.

1.1 Procedural notes

1. To use the AbsoluteIDQ® p180 kit for metabolomics research successfully, all components of the kit should be understood thoroughly.
2. Any deviation from the assay procedure may affect the results.
3. In order not to waste valuable sample material, make sure that you are familiar with the MetIDQ™ software and that your analytical system meets the requirements before running the assay. If necessary, contact biocrates customer support about the starter kit option to help you.
4. Re-using standard solutions or quality control (QC) material is not recommended. Only use original kit reagents and contents for your assay.
5. If you have any technical questions do not hesitate to contact customer support.
support@biocrates.com.

1.2 Limitations of the AbsoluteIDQ® p180 kit

The kit offers a device for metabolomics research. The analytical focus of the kit lies on measuring a very broad spectrum of physiological metabolites in an easy, reliable and robust way. To overcome limitations of a pure flow injection (FIA-) ESI-MS/MS detection as in the AbsoluteIDQ® p150 kit, the p180 kit adds an LC-ESI-MS/MS run to expand the range of analytes that can be quantified. However, with respect to the analytes that are still quantified in the FIA part of the assay, the kit was developed with the goal of finding a reasonable compromise between analytical speed, robustness and quantitative measures. Therefore, the FIA procedure of the p180 kit cannot detect all members of a metabolite class that are biochemically or medically important in an analytically valid manner. However, to deliver the broadest information possible, biocrates has incorporated several analytes that do not meet the general validation criteria specified by the FDA Guidance for Industry - Bioanalytical Method Validation. These analytes exhibited robust values with good variance criteria and are discussed in the sections overleaf. Furthermore, in terms of quantification, the lipids and a subset of acylcarnitines are classified as "relative quantitative", because specific standards and internal standards were not commercially available, and a verification of the accuracy was therefore not possible. In addition, concentration values of many "relative quantitative" analytes represent total concentrations of isobaric compounds. MetIDQ® displays a corresponding short name with the total lengths of side chains and the total number of double bonds. From biocrates' experience during development and beta testing, these general conditions are known to cause anomalous results:

- Contamination of the instrumentation: autosampler, capillaries, tubing, etc.
- Incomplete reconstitution of standards
- Suboptimal calibrated and tuned mass spectrometer
- Imprecise pipetting of sample or control material
- Inhomogeneous sample material (for example, clotted plasma)
- Improper storage of sample material before running the assay

1.3 Mass spectrometers

In the table below you find a categorization of the instruments with the validation status. The categories are described underneath. With the SCIEX 4000 series, only the normal HPLC method can be used. With the other instruments, HPLC and UHPLC can be applied.

Instrument	Status (human plasma)
SCIEX 3200 series	— ¹⁾
SCIEX 4000 series (HPLC only)	V
SCIEX 4500 series	F
SCIEX 5500 series	A
SCIEX 6500 series	A
SCIEX 6500+ series	A
Waters Xevo TQ MS	V
Waters Xevo TO-S	V
Waters Xevo TQ-S micro	V
Waters Xevo TQ-XS	A
Thermo TSQ Vantage	V
Agilent 6470	V

1) sensitivity not sufficient

F = Feasibility tests successfully completed. Acquisition methods available.

A = Adapted. Partial validation (intraday precision)

V = Fully validated. Accuracy and precision tested (intraday/interday), selectivity and stability tested.

1.4 Calibration standards

With the AbsoluteIDQ® p180 kit, amino acids and biogenic amines are quantified by stable isotopes in the LC-MS/MS mode. Therefore, in contrast to the p150 kit and in contrast to analytes that are detected in FIA mode, seven different concentration levels of (external) standard mixes are provided as lyophilized material with the p180 kit. The dissolved standards are used as calibration standards in order to perform the quantification in the MS-specific software. Table 1 and Table 2 show the concentrations of the different levels in the standard mix.

Note: Please make sure that the standards are completely dissolved before pipetting them onto the AbsoluteIDQ® kit plate.

Table 1: Concentration of amino acid standards at different calibration standard levels (µM)

Analyte	Calibration standard level						
MetIDQ Short name	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
HPLC: U(H)PLC:	20000611 437301	20000612 437314	20000613 437328	20000614 437331	20000615 437345	20000616 437359	20000617 437362
Ala	20	40	200	400	800	1200	1600
Arg	5	10	50	100	200	300	400
Asn	5	10	50	100	200	300	400
Asp	5	10	50	100	200	300	400
Cit	5	10	50	100	200	300	400
Gin	20	40	200	400	800	1200	1600
Glu	10	20	100	200	400	600	800
Gly	25	50	250	500	1000	1500	2000
His	5	10	50	100	200	300	400
Ile	5	10	50	100	200	300	400
Leu	5	10	50	100	200	300	400
Lys	10	20	100	200	400	600	800
Met	5	10	50	100	200	300	400
Orn	5	10	50	100	200	300	400
Phe	5	10	50	100	200	300	400
Pro	10	20	100	200	400	600	800
Ser	5	10	50	100	200	300	400
Thr	5	10	50	100	200	300	400
Trp	5	10	50	100	200	300	400
Tyr	5	10	50	100	200	300	400
Val	10	20	100	200	400	600	800

Table 2: Concentration of biogenic amine standards at different calibration standard levels (µM)

Analyte	Calibration standard level						
MetIDQ Short name	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
HPLC: U(H)PLC:	20000611 437301	20000612 437314	20000613 437328	20000614 437331	20000615 437345	20000616 437359	20000617 437362
Ac-Orn	0.5	1	5	10	20	30	40
ADMA	0.25	0.5	2.5	5	10	15	20
SDMA (HPLC) SDMA (UHPLC)	1 0.1	2 0.2	10 1	20 2	40 4	60 6	80 8
alpha-AAA	1	2	10	20	40	60	80
Carnosine	0.5	1	5	10	20	30	40
Creatinine	10	20	100	200	400	600	800
DOPA	0.5	1	5	10	20	30	40
Dopamine	1	2	10	20	40	60	80
Histamine	1	2	10	20	40	60	80
Kynurenine	1	2	10	20	40	60	80
Met-SO	1	2	10	20	40	60	80
Nitro-Tyr ¹⁾	1	2	10	20	40	60	80
c4-OH-Pro	1	2	10	20	40	60	80
t4-OH-Pro ²⁾	1	2	10	20	40	60	80
PEA	0.1	0.2	1	2	4	6	8
Putrescine	0.1	0.2	1	2	4	6	8
Sarcosine ³⁾	1	2	10	20	40	60	80
Serotonin	0.1	0.2	1	2	4	6	8
Spermidine	0.25	0.5	2.5	5	10	15	20
Spermine	0.25	0.5	2.5	5	10	15	20
Taurine	2.5	5	25	50	100	150	200

1) not analyzed with Thermo TSQ Vantage

3) only analyzed with the UHPLC method

2) not validated yet

1.5 Quality control

There are three different quality control levels (QCs) to be positioned on the plate as described in the user manual. The controls represent lyophilized human plasma samples (anticoagulant: EDTA) with respectively low, medium and high (spiked) concentration levels. Results are displayed in the MetVAL module of MetIDQ™ and are also shown in the results table of the MetSTAT module. In MetVAL, some of the quality controls often appear with an invalid status. In most cases, this happens due to single outliers. Due to the high number of analytes that are measured with the kit, unspecific analytical variations may appear leading to single outliers.

2 Analytical performance characteristics

2.1 Overview

The tables overleaf show all analytes of the AbsoluteIDQ® p180 kit with analyte classification and quantification parameters. On this page you find an overview and explanations for the tables overleaf.

Table	Compounds	Mode	MetIDQ - OP Code
Table 3	Acylcarnitines	FIA	KIT3
Table 4	Amino acids	LC	KIT2
Table 5	Biogenic amines	LC	KIT2
Table 6	Glycerophospholipids	FIA	KIT3
Table 7	Sphingolipids	FIA	KIT3
Table 8	Sugars	FIA	KIT3

Issue	Explanation
Quantitative (quant.)	Validation criteria fulfilled: accuracy = 80-120%, precision (CV) < 15%
Quantitative with restrictions	Also "quantitative", but poorer precision expected: CV = 15-30%
Relative quantitative (rel. quant.)	Precision criteria fulfilled (CV < 15%), but accuracy not verified: - standards for accuracy testing not available - internal standards for quantification are chemically different
LOD	Limit of detection
LLOQ	Lower limit of quantification
ULOQ	Upper limit of quantification

Table 3: Acylcarnitines

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (μM)	LLOQ (μM)	ULOQ (μM)
1	C0	Carnitine	X		4	5	120
2	C2	Acetylcarnitine	X		0.15	0.4	35
3	C3	Propionylcarnitine*	X		0.08	0.4	15
4	C3:1	Propenoylcarnitine		X	0.03		
5	C3-OH	Hydroxypropionylcarnitine"		X	0.05		
6	C4	Butyrylcarnitine*	X		0.03	0.4	12
7	C4:1	Butenylcarnitine		X	0.03		
8	C4-OH (C3-DC)	Hydroxybutyrylcarnitine*		X	0.09		
9	C5	Valerylcarnitine*	X		0.04	0.4	12
10	C5:1	Tiglylcarnitine		X	0.04		
11	C5:1-DC	Glutaconylcarnitine		X	0.015		
12	C5-DC (C6-OH)	Glutaryl carnitine* (Hydroxyhexanoylcarnitine)		X	0.035		
13	C5-M-DC	Methylglutaryl carnitine		X	0.06		
14	C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine* (Methylmalonylcarnitine)		X	0.1		
15	C6 (C4:1-DC)	Hexanoylcarnitine* (Fumaryl carnitine)	X		0.08	0.2	6
16	C6:1	Hexenoylcarnitine		X	0.035		
17	C7-DC	Pimelylcarnitine*		X	0.035		
18	C8	Octanoylcarnitine*	X		0.17	0.2	8
19	C9	Nonaylcarnitine*		X	0.04		
20	C10	Decanoylcarnitine*	X		0.16	0.3	6
21	C10:1	Decenoylcarnitine*		X	0.12		
22	C10:2	Decadienyl carnitine		X	0.04		
23	C12	Dodecanoylcarnitine"	X		0.057	0.4	12
24	C12:1	Dodecenoylcarnitine*		X	0.2		
25	C12-DC	Dodecanedioyl carnitine*		X	0.2		
26	C14	Tetradecanoylcarnitine*	X		0.03	0.4	6
27	C14:1	Tetradecenoylcarnitine"		X	0.015		
28	C14:1-OH	Hydroxytetradecanoylcarnitine*		X	0.015		
29	C14:2	Tetradecadienyl carnitine		X	0.012		

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
30	C14:2-OH	Hydroxytetradecadienyl-carnitine		X	0.015		
31	C16	Hexadecanoylcarnitine*	X		0.018	0.4	12
32	C16:1	Hexadecenoylcarnitine*		X	0.06		
33	C16:1-OH	Hydroxyhexadecenoylcarnitine*		X	0.02		
34	C16:2	Hexadecadienylcarnitine		X	0.008		
35	C16:2-OH	Hydroxyhexadecadienyl-carnitine		X	0.03		
36	C16-OH	Hydroxyhexadecanoylcarnitine*		X	0.015		
37	C18	Octadecanoylcarnitine*	X		0.02	0.4	6
38	C18:1	Octadecenoylcarnitine*		X	0.04		
39	C18:1-OH	Hydroxyoctadecenoylcarnitine		X	0.023		
40	C18:2	Octadecadienylcarnitine		X	0.009		

* Concentrations are isotope corrected

Table 4: Amino acids

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
44	Ala	Alanine	X		1	20	1600
45	Arg	Arginine	X		0.5	5	400
46	Asn	Asparagine	X		1.5	5	400
47	Asp	Aspartate	X	X ¹⁾	1.5	5	400
48	Cit	Citrulline	X		1	5	400
49	Gln	Glutamine	X		1.5	20	1600
50	Glu	Glutamate	X		2	10	800
51	Gly	Glycine	X		0.5	25	2000
52	His	Histidine	X		0.5	5	400
53	Ile	Isoleucine	X		1.5	5	400
54	Leu	Leucine	X	X ²⁾	1.5 ³⁾	50 ⁴⁾	400
55	Lys	Lysine	X		0.5	10	800
56	Met	Methionine	X		0.1	5	400
57	Orn	Ornithine	X		0.5	5	400
58	Phe	Phenylalanine	X		0.1	5	400
59	Pro	Proline	X		1	10	800
60	Ser	Serine	X		1	5	400
61	Thr	Threonine	X		0.5	5	400

1) only SCIEX mass spectrometers

2) only Waters Xevo® TO-S

3) Waters mass spectrometers: LOD = 20 µM

4) LLOQ at Cal3 level

Table 5: Biogenic amines

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
62	Ac-Orn	Acetylornithine		X ²⁾	0.15	1	40
63	ADMA	Asymmetric dimethylarginine		X ²⁾	0.08	0.25	20
64	SDMA (HPLC)	Symmetric dimethylarginine	HPLC	X	0.3	2	80
	SDMA (UHPLC)		UHPLC	X	0.03	0.1	8
65	total DMA ¹⁾	Total dimethylarginine	X		0.1	1.25	100
66	alpha-AAA	alpha-Aminoadipic acid		X	0.3	1	80
67	Carnosine	Carnosine	X		0.1	0.5	40
68	Creatinine	Creatinine	X	X ²⁾	1	10	800
69	DOPA	Dihydroxyphenylalanine		X ²⁾	0.17	0.5	40
70	Dopamine	Dopamine		X ²⁾	0.3	1	80
71	Histamine	Histamine		X	0.3	1	80
72	Kynurenine	Kynurenine		X	0.3	1	80
73	Met-SO	Methionine sulfoxide	X		0.3	10	80
74	Nitro-Tyr ³⁾	Nitrotyrosine		X	0.3	1	80
75	c4-OH-Pro	cis-4-Hydroxyproline	X		0.1	10	80
76	t4-OH-Pro ⁴⁾	trans-4-Hydroxyproline	X			0.1	10
77	PEA	Phenylethylamine		X	0.03	0.1	8
78	Putrescine	Putrescine	X		0.03	0.1	88
79	Sarcosine ⁵⁾	Sarcosine	X		0.3	1	80
80	Serotonin	Serotonin	X		0.03	0.1	8
81	Spermidine	Spermidine	X		0.08	0.25	20
82	Spermine	Spermine		X	0.08	0.25	20
83	Taurine	Taurine	X		0.5	2.5	200

- 1) not analyzed with the UHPLC method
 2) only SCIEX mass spectrometers
 3) not analyzed with Thermo TSQ Vantage
 4) not validated yet
 5) only analyzed with the UHPLC method

Table 6: Glycerophospholipids

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
84	lysoPC a C14:0	lysoPhosphatidylcholine acyl C14:0		X	5		
85	lysoPC a C16:0	lysoPhosphatidylcholine acyl C16:0*		X	0.12		
86	lysoPC a C16:1	lysoPhosphatidylcholine acyl C16:1		X	0.07		
87	lysoPC a C17:0	lysoPhosphatidylcholine acyl C17:0		X	0.05		
88	lysoPC a C18:0	lysoPhosphatidylcholine acyl C18:0*		X	0.05		
89	lysoPC a C18:1	lysoPhosphatidylcholine acyl C18:1*		X	0.1		
90	lysoPC a C18:2	lysoPhosphatidylcholine acyl C18:2		X	0.1		
91	lysoPC a C20:3	lysoPhosphatidylcholine acyl C20:3*		X	0.2		
92	lysoPC a C20:4	lysoPhosphatidylcholine acyl C20:4		X	0.02		
93	lysoPC a C24:0	lysoPhosphatidylcholine acyl C24:0		X	1.3		
94	lysoPC a C26:0	lysoPhosphatidylcholine acyl C26:0*		X	0.5		
95	lysoPC a C26:1	lysoPhosphatidylcholine acyl C26:1		X	4		
96	lysoPC a C28:0	lysoPhosphatidylcholine acyl C28:0*		X	0.3		
97	lysoPC a C28:1	lysoPhosphatidylcholine acyl C28:1		X	0.15		
98	PC aa C24:0	Phosphatidylcholine diacyl C24:0		X	0.1		
99	PC aa C26:0	Phosphatidylcholine diacyl C26:0		X	1.4		
100	PC aa C28:1	Phosphatidylcholine diacyl C28:1		X	0.04		
101	PC aa C30:0	Phosphatidylcholine diacyl C30:0*		X	0.2		
102	PC aa C30:2**	Phosphatidylcholine diacyl C30:2*		X	0.006		
103	PC aa C32:0	Phosphatidylcholine diacyl C32:0*		X	0.04		
104	PC aa C32:1	Phosphatidylcholine diacyl C32:1*		X	0.06		
105	PC aa C32:2	Phosphatidylcholine diacyl C32:2*		X	0.03		
106	PC aa C32:3	Phosphatidylcholine diacyl C32:3		X	0.008		
107	PC aa C34:1	Phosphatidylcholine diacyl C34:1*		X	0.06		
108	PC aa C34:2	Phosphatidylcholine diacyl C34:2*		X	0.1		
109	PC aa C34:3	Phosphatidylcholine diacyl C34:3*		X	0.01		
110	PC aa C34:4	Phosphatidylcholine diacyl C34:4		X	0.006		
111	PC aa C36:0	Phosphatidylcholine diacyl C36:0*		X	0.2		
112	PC aa C36:1	Phosphatidylcholine diacyl C36:1*		X	0.03		
113	PC aa C36:2	Phosphatidylcholine diacyl C36:2*		X	0.15		
114	PC aa C36:3	Phosphatidylcholine diacyl C36:3*		X	0.04		
115	PC aa C36:4	Phosphatidylcholine diacyl C36:4*		X	0.04		

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
116	PC aa C36:5	Phosphatidylcholine diacyl C36:5*		X	0.01		
117	PC aa C36:6	Phosphatidylcholine diacyl C36:6*		X	0.015		
118	PC aa C38:0	Phosphatidylcholine diacyl C38:0*		X	0.2		
119	PC aa C38:1**	Phosphatidylcholine diacyl C38:1*		X	0.08		
120	PC aa C38:3	Phosphatidylcholine diacyl C38:3*		X	0.04		
121	PC aa C38:4	Phosphatidylcholine diacyl C38:4*		X	0.03		
122	PC aa C38:5	Phosphatidylcholine diacyl C38:5*		X	0.015		
123	PC aa C38:6	Phosphatidylcholine diacyl C38:6*		X	0.02		
124	PC aa C40:1	Phosphatidylcholine diacyl C40:1*		X	0.4		
125	PC aa C40:2	Phosphatidylcholine diacyl C40:2*		X	0.02		
126	PC aa C40:3	Phosphatidylcholine diacyl C40:3*		X	0.006		
127	PC aa C40:4	Phosphatidylcholine diacyl C40:4*		X	0.01		
128	PC aa C40:5	Phosphatidylcholine diacyl C40:5*		X	0.04		
129	PC aa C40:6	Phosphatidylcholine diacyl C40:6*		X	1.2		
130	PC aa C42:0	Phosphatidylcholine diacyl C42:0*		X	0.05		
131	PC aa C42:1	Phosphatidylcholine diacyl C42:1*		X	0.008		
132	PC aa C42:2	Phosphatidylcholine diacyl C42:2*		X	0.006		
133	PC aa C42:4	Phosphatidylcholine diacyl C42:4*		X	0.006		
134	PC aa C42:5	Phosphatidylcholine diacyl C42:5*		X	0.05		
135	PC aa C42:6	Phosphatidylcholine diacyl C42:6*		X	0.3		
136	PC ae C30:0	Phosphatidylcholine acyl-alkyl C30:0*		X	0.15		
137	PC ae C30:1	Phosphatidylcholine acyl-alkyl C30:1*		X	0.02		
138	PC ae C30:2	Phosphatidylcholine acyl-alkyl C30:2		X	0.57		
139	PC ae C32:1	Phosphatidylcholine acyl-alkyl C32:1*		X	0.009		
140	PC ae C32:2	Phosphatidylcholine acyl-alkyl C32:2		X	0.02		
141	PC ae C34:0	Phosphatidylcholine acyl-alkyl C34:0*		X	0.017		
142	PC ae C34:1	Phosphatidylcholine acyl-alkyl C34:1*		X	0.012		
143	PC ae C34:2	Phosphatidylcholine acyl-alkyl C34:2*		X	0.01		
144	PC ae C34:3	Phosphatidylcholine acyl-alkyl C34:3		X	0.015		
145	PC ae C36:0	Phosphatidylcholine acyl-alkyl C36:0*		X	0.12		
146	PC ae C36:1	Phosphatidylcholine acyl-alkyl C36:1*		X	0.03		
147	PC ae C36:2	Phosphatidylcholine acyl-alkyl C36:2*		X	0.01		
148	PC ae C36:3	Phosphatidylcholine acyl-alkyl C36:3*		X	0.007		

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
149	PC ae 036:4	Phosphatidylcholine acyl-alkyl C36:4*		X	0.013		
150	PC ae 036:5	Phosphatidylcholine acyl-alkyl 036:5		X	0.012		
151	PC ae 038:0	Phosphatidylcholine acyl-alkyl C38:0*		X	0.066		
152	PC ae C38:1	Phosphatidylcholine acyl-alkyl C38:1*		X	0.015		
153	PC ae C38:2	Phosphatidylcholine acyl-alkyl C38:2*		X	0.018		
154	PC ae C38:3	Phosphatidylcholine acyl-alkyl C38:3*		X	0.01		
155	PC ae C38:4	Phosphatidylcholine acyl-alkyl C38:4"		X	0.015		
156	PC ae C38:5	Phosphatidylcholine acyl-alkyl C38:5*		X	0.01		
157	PC ae C38:6	Phosphatidylcholine acyl-alkyl C38:6"		X	0.03		
158	PC ae C40:1	Phosphatidylcholine acyl-alkyl C40:1*		X	0.06		
159	PC ae C40:2	Phosphatidylcholine acyl-alkyl C40:2*		X	0.01		
160	PC ae C40:3	Phosphatidylcholine acyl-alkyl C40:3*		X	0.015		
161	PC ae C40:4	Phosphatidylcholine acyl-alkyl C40:4*		X	0.1		
162	PC ae C40:5	Phosphatidylcholine acyl-alkyl C40:5*		X	0.006		
163	PC ae C40:6	Phosphatidylcholine acyl-alkyl C40:6*		X	0.025		
164	PC ae C42:0	Phosphatidylcholine acyl-alkyl C42:0*		X	0.4		
165	PC ae C42:1	Phosphatidylcholine acyl-alkyl C42:1*		X	0.03		
166	PC ae C42:2	Phosphatidylcholine acyl-alkyl C42:2*		X	0.006		
167	PC ae C42:3	Phosphatidylcholine acyl-alkyl C42:3*		X	0.006		
168	PC ae C42:4	Phosphatidylcholine acyl-alkyl C42:4*		X	0.3		
169	PC ae C42:5	Phosphatidylcholine acyl-alkyl C42:5		X	1.3		
170	PC ae C44:3	Phosphatidylcholine acyl-alkyl C44:3*		X	0.006		
171	PC ae C44:4	Phosphatidylcholine acyl-alkyl C44:4*		X	0.01		
172	PC ae C44:5	Phosphatidylcholine acyl-alkyl C44:5*		X	0.02		
173	PC ae C44:6	Phosphatidylcholine acyl-alkyl C44:6*		X	0.09		

Table 7: Glycerophospholipids

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
174	SM (OH) C14:1	Hydroxysphingomyeline C14:1*		X	0.025		
175	SM C16:0	Sphingomyeline C16:0*		X	0.03		
176	SM C16:1	Sphingomyeline C16:1		X	0.01		
177	SM (OH) C16:1	Hydroxysphingomyeline C16:1*		X	0.012		
178	SM C18:0	Sphingomyeline 018:0"		X	0.07		
179	SM C18:1	Sphingomyeline C18:1*		X	0.01		
180	SM C20:2	Sphingomyeline 020:2*		X	0.005		
181	SM C22:3**	Sphingomyeline C22:3*		X	0.01		
182	SM (OH) 022:1	Hydroxysphingomyeline 022:1*		X	0.015		
183	SM (OH) 022:2	Hydroxysphingomyeline C22:2*		X	0.01		
184	SM C24:0	Sphingomyeline 024:0*		X	0.13		
185	SM C24:1	Sphingomyeline C24:1*		X	0.035		
186	SM (OH) C24:1	Hydroxysphingomyeline 024:1*		X	0.01		
187	SM C26:0	Sphingomyeline C26:0*		X	0.015		
188	SM C26:1	Sphingomyeline C26:1*		X	0.006		

* concentrations are isotope corrected

** not analyzed with Waters mass spectrometers and Thermo TSQ Vantage

Table 8: Sugars

	Analyte		Analyte classification		Quantification range		
	MetIDQ short name	Biochemical name	Quant.	Rel. quant.	LOD (µM)	LLOQ (µM)	ULOQ (µM)
189	H1 ¹⁾	Hexose	X		20	200	30000

1) The performance of hexose is critical on Waters Xevo TQ-S instruments and does often show a reduced sensitivity and signal stability. The performance depends on the used TQ-S instrument. Please check the performance of H1 carefully.

Table 9 shows the analytes that gave restricted validation parameters during product validation of the LC-MS/MS part (KIT2-0-x). Most of those analytes had difficulties to fulfill the 15% criteria for inter-day precision as required by the FDA Guidance for Industry - Bioanalytical Method Validation. Therefore, it is recommended to process samples of one study on one or more kit plates in the same analytical run. Further details of the validation experiments are discussed in sections 2.2 – 2.6 of this document.

Table 9: Analytes with restricted validation parameters (for human plasma)

Analyte		Restricted validation criteria
MetIDQ short name	Biochemical name	
Asp	Aspartate	Inter-day precision
Ac-Orn	Acetylorntithine	Inter-day accuracy
ADMA	Asymmetric dimethylarginine	Inter-day precision
SDMA	Symmetric dimethylarginine	Inter-day precision
alpha-AAA	alpha-Aminoadipic acid	Inter-day Precision + Inter-/Intra-day Accuracy
Histamine	Histamine	Inter-day Precision + Inter-/Infra-day Accuracy
Kynurenine	Kynurenine	Inter-day Precision + Inter-/Infra-day Accuracy
Met-SO	Methionine sulfoxide	Inter-day Precision + Inter-day Accuracy
Nitro-Tyr ¹⁾	Nitrotyrosine	Inter-/Intra-day Accuracy
PEA	Phenylethylamine	Inter-/Intra-day Accuracy
Spermidine	Spermidine	Inter-day Precision + Intra-day Accuracy
Spermine	Spermine	Inter-day Precision + Inter-/Intra-day Accuracy
Creatinine	Creatinine	Inter-day Precision + Inter-Antra-day Accuracy (Waters only)
DOPA	Dihydroxyphenylalanine	Inter-day Precision + Inter-/Intra-day Accuracy
Dopamine	Dopamine	Inter-/Intl-a-day Precision + Inter-Antra-day Accuracy

1) Not analyzed with Thermo TSQ Vantage

2.2 Specificity

The analytes and the corresponding MRM pairs were tested for selectivity and specificity in case there were corresponding standards available. For some analytes measured in the FIA mode, potential interferences by other compounds were detected. Table 10 summarizes relevant interferences with respect to semi-quantitative analytes. A detailed list with all potential isobaric and isomeric lipids is available. Please contact the support: biocrates.com/support.

Table 10: Potential interferences of relative-quantitative analytes (FIA part)

Analyte	Analyte classification		Interference by	Effect	Remarks
	MetIDQ short name	Quant. Rel. quant.			
lysoPC a C18:0		X	PC aa 036:0	11% of the concentration of PC aa C36:0 is detectable as part of the lysoPC a C18:0 concentration.	In normal human plasma, the effect is not great since conc. of lysoPC a C18:0 is approx. 8 times higher than conc. of PC aa C36:0.
PC ae C38:6		X	PC aa C36:0	7% of the concentration of PC aa C36:0 is detectable as part of the PC ae C38:6 concentration.	Relevant in normal human plasma since both concentrations are in the same µM range.

Measuring the zero samples in the LC mode, the analyte Histamine does often not result in zero counts. However, this is not relevant as far as it does not concern physiological concentrations in plasma. Similar results are achieved for PEA and Spermidine although this occurs only occasionally and depends on the system condition. The isobaric analyte pair Leu/Ile exhibits some well characterized limitations regarding selectivity that are summarized in Table 11.

Table 11: Potential interferences of LC-MS/MS-derived analytes

Analyte	Analyte classification		Interference by	Effect	Remarks
	MetIDQ short name	Quant. Rel. quant.			
Leu		X	Ile	10% of the concentration of Isoleucine is detectable as part of the Leu-cine concentration.	In human plasma, the concentration of Leucine is generally 1.5 - 2 times higher than the concentration of Isoleucine.
Ile		X	Leu	3% of the concentration of Leucine is detectable as part of the Iso-leucine concentration.	Relevant in normal human plasma since Leucine is generally 1.5 - 2 times higher than the concentration of Isoleucine.

2.3 Calibration, precision and accuracy

2.3.1 LC-MS/MS part of the AbsoluteIDQ® p180 kit

To determine the validation parameters of the LC-MS/MS part, calibration curves were generated using a standard mix with all amino acids and biogenic amines that are covered by the LC part at 7 different concentration levels.

Linear or quadratic regressions were performed using data of six repetitive preparations by plotting the mean concentrations of the replicates against the theoretical amount. R^2 values were calculated, whereby most analytes exhibited values of $R^2 \geq 0.990$. Table 12 and Table 13 show the regression parameters for amino acids and biogenic amines, respectively. The values are based on the SCIEX 4000 QTRAP® and are similar for all other MS platforms.

Table 12: Regression and precision of amino acids

Analyte	Regression	Calibration standard level (CV)						
MetIDQ short name	R^2	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
Ala	0.9982	7%	5%	6%	5%	6%	5%	5%
Arg	0.9958	11%	10%	10%	12%	10%	8%	12%
Asn	0.9947	8%	10%	8%	10%	7%	9%	10%
Asp	0.9971	6%	8%	6%	6%	9%	4%	9%
Cit	0.9968	12%	8%	7%	7%	9%	11%	7%
Gin	0.9936	10%	14%	13%	16%	11%	8%	10%
Glu	0.9956	8%	11%	3%	12%	8%	11%	8%
Gly	0.9944	10%	12%	14%	12%	13%	5%	8%
His	0.9951	9%	14%	12%	14%	9%	6%	10%
Ile	0.9954	8%	11%	12%	13%	9%	5%	8%
Leu	0.9958	28%	27%	13%	10%	8%	5%	6%
Lys	0.9918	10%	14%	16%	14%	15%	8%	12%
Met	0.9930	10%	16%	18%	15%	13%	5%	11%
Orn	0.9913	13%	12%	16%	14%	14%	10%	12%
Phe	0.9954	8%	12%	12%	12%	11%	5%	10%
Pro	0.9965	7%	8%	8%	13%	6%	8%	9%
Ser	0.9914	17%	18%	13%	14%	13%	11%	13%
Thr	0.9992	7%	6%	4%	4%	4%	3%	4%
Trp	0.9952	7%	11%	13%	11%	10%	8%	11%
Tyr	0.9954	9%	13%	10%	14%	9%	8%	9%
Val	0.9938	10%	13%	13%	13%	11%	7%	10%

Table 13: Regression and precision of biogenic amines

Analyte	Regression	Calibration standard level (CV)						
MetIDQ short name	R ²	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
Ac-Orn	0.9924	26%	16%	12%	14%	11%	8%	9%
ADMA	0.9972	15%	14%	7%	5%	3%	9%	10%
SDMA	0.9965	36%	16%	6%	6%	5%	11%	12%
total DMA ¹⁾	0.9970	8%	12%	8%	6%	3%	10%	13%
alpha-AAA	0.9914	17%	12%	12%	12%	13%	8%	10%
Carnosine	0.9954	7%	11%	13%	12%	10%	7%	8%
Creatinine	0.9998	2%	2%	1%	2%	2%	3%	2%
DOPA	0.9978	4%	9%	8%	5%	5%	5%	5%
Dopamine	0.9989	4%	5%	1%	3%	5%	3%	4%
Histamine	0.9959	4%	10%	11%	13%	7%	8%	10%
Kynurenine	0.9915	11%	15%	10%	14%	10%	9%	9%
Met-SO	0.9938	16%	26%	15%	12%	13%	6%	8%
Nitro-Tyr ²⁾	0.9960	4%	13%	12%	13%	9%	6%	8%
c4-OH-Pro	0.9970	20%	22%	11%	12%	6%	5%	4%
PEA	0.9990	4%	5%	2%	3%	5%	5%	4%
Putrescine	0.9978	6%	8%	4%	4%	3%	7%	7%
Serotonin	0.9985	9%	8%	3%	4%	6%	5%	6%
Spermidine	0.9957	1%	2%	1%	3%	5%	5%	10%
Spermine	0.9904	1%	1%	3%	3%	6%	8%	8%
Taurine	0.9989	4%	7%	4%	4%	5%	7%	7%

1) Not analyzed with the UHPLC method

2) Not analyzed with Thermo TSQ Vantage

The accuracy of the measurements was determined for each calibration level, as summarized in Table 14 and Table 15 for amino acids and biogenic amines, respectively. All analyses exhibited valid accuracies in the range of 80 – 115%. The values are based on analyzes performed with a SCIEX 4000 QTRAP® and are similar for all other MS platforms.

Table 14: Accuracy of amino acids

Analyte	Calibration standard level (accuracy)						
MetIDQ short name	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
Ala	100%	101%	100%	99%	98%	103%	99%
Arg	97%	96%	107%	104%	95%	98%	104%
Asn	96%	97%	108%	105%	92%	102%	101%
Asp	103%	98%	95%	105%	98%	101%	100%
Cit	97%	103%	99%	100%	101%	98%	101%
Gln	97%	96%	107%	104%	94%	101%	100%
Glu	97%	102%	102%	100%	97%	103%	99%
Gly	97%	92%	111%	106%	95%	96%	103%
His	99%	95%	106%	105%	96%	99%	101%
Ile	96%	95%	111%	104%	96%	97%	102%
Leu	82%	110%	108%	105%	95%	98%	102%
Lys	91%	99%	112%	104%	93%	99%	102%
Met	100%	92%	108%	104%	97%	97%	102%
Orn	95%	95%	111%	105%	93%	99%	102%
Phe	98%	96%	108%	103%	96%	99%	101%
Pro	95%	99%	108%	102%	97%	97%	102%
Ser	95%	97%	109%	103%	95%	99%	101%
Thr	99%	98%	104%	99%	99%	101%	100%
Trp	99%	95%	108%	102%	97%	99%	102%
Tyr	99%	95%	106%	102%	96%	100%	101%
Val	98%	93%	110%	103%	95%	99%	101%

Table 15: Accuracy of biogenic amines

Analyte	Calibration standard level (accuracy)						
MetIDQ short name	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7
Ac-Orn	90%	96%	112%	105%	94%	100%	101%
ADMA	101%	99%	99%	100%	102%	98%	101%
SDMA	98%	104%	96%	102%	100%	101%	100%
total DMA ¹⁾	100%	100%	97%	105%	98%	99%	102%
alpha-AAA	98%	94%	105%	104%	97%	98%	101%
Carnosine	99%	94%	107%	104%	95%	100%	101%
Creatinine	101%	99%	100%	100%	100%	100%	100%
DOPA	105%	96%	96%	104%	97%	102%	99%
Dopamine	109%	97%	91%	100%	101%	102%	99%
Histamine	102%	95%	101%	105%	96%	99%	101%
Kynurenine	99%	94%	109%	101%	96%	100%	101%
Met-SO	90%	100%	112%	101%	98%	96%	102%
Nitro-Tyr ²⁾	102%	96%	103%	101%	98%	101%	100%
c4-OH-Pro	89%	101%	109%	104%	96%	99%	101%
PEA	103%	95%	103%	99%	101%	98%	101%
Putrescine	96%	101%	105%	102%	96%	101%	100%
Serotonin	100%	99%	101%	101%	99%	100%	100%
Spermidine	117%	91%	91%	97%	103%	102%	98%
Spermine	92%	88%	105%	107%	102%	89%	99%
Taurine	97%	100%	105%	100%	98%	99%	102%

1) Not analyzed with the UHPLC method

2) Not analyzed with Thermo TSQ Vantage

The precision and the accuracy were also determined at different concentration levels (diluted and spiked plasma). For the amino acids, two levels of diluted plasma (1:5 and 1:2 in PBS with 4% HSA), non-spiked plasma and three spiked plasma (with calibration standard levels 2, 3 and 5) were tested. The diluted plasma levels were introduced because non-spiked plasma showed much higher concentration values than the LLOQs. The results for the intra-day experiments are shown in Table 16. For the biogenic amines, due to the low abundance in non-spiked plasma, three spike levels (with calibration standard levels 2, 3 and 5) were tested. The results are summarized in Table 17.

Table 16: Intra-day precision and accuracy of amino acids in matrix (human pool plasma)

Analyte	Plasma 1:5		Plasma 1:2		Plasma non-spiked		Plasma spiked Cal2		Plasma spiked Cal3		Plasma spiked Cal5	
MetIDQ short name	CV	Acc. ¹⁾	CV	Acc.	CV	Acc.	CV	Acc.	CV	Acc.	CV	Acc.
Ala	5%	106%	3%	100%	4%	--	4%	103%	5%	104%	5%	106%
Arg	4%	102%	5%	96%	9%	--	8%	98%	9%	104%	14%	98%
Asn	-- *		4%	90%	10%	--	10%	102%	6%	95%	5%	93%
Asp	-- *		16%	94%	7%	--	9%	104%	6%	104%	13%	111%
Cit	10%	111%	9%	104%	7%	--	8%	95%	7%	97%	5%	103%
Gln	11%	96%	6%	97%	9%	--	7%	99%	6%	106%	11%	103%
Glu	9%	102%	6%	100%	6%	--	2%	95%	7%	99%	10%	105%
Gly	10%	99%	4%	96%	9%	--	7%	107%	10%	114%	8%	102%
His	8%	97%	3%	91%	7%	--	5%	101%	7%	108%	12%	101%
Ile	12%	92%	7%	89%	7%	--	3%	103%	5%	110%	11%	106%
Leu	-- *		6%	89%	8%	--	5%	101%	5%	111%	9%	114%
Lys	8%	107%	6%	94%	8%	--	6%	102%	5%	106%	13%	106%
Met	-- *		12%	85%	13%	--	6%	104%	6%	114%	11%	101%
Orn	7%	101%	10%	90%	8%	--	6%	101%	6%	102%	13%	101%
Phe	11%	99%	5%	92%	9%	--	3%	102%	5%	106%	9%	95%
Pro	10%	100%	4%	94%	10%	--	6%	98%	5%	102%	9%	87%
Ser	5%	94%	7%	92%	10%	--	7%	101%	9%	108%	9%	99%
Thr	6%	109%	7%	102%	4%	--	5%	102%	3%	102%	4%	102%
Trp	10%	98%	5%	94%	6%	--	6%	101%	5%	104%	11%	92%
Tyr	14%	93%	6%	95%	11%	--	5%	96%	7%	104%	7%	94%
Val	8%	109%	6%	96%	8%	--	5%	103%	4%	106%	10%	98%

1) Acc. = accuracy

* detected concentrations below LLOQ

Table 17: Intra-day precision and accuracy of biogenic amines in matrix (human pool plasma)

Analyte	Plasma non-spiked		Plasma spiked Cal2		Plasma spiked Cal3		Plasma spiked Cal5	
MetIDQ short name	CV	Accuracy	CV	Accuracy	CV	Accuracy	CV	Accuracy
Ac-Orn	11%	--	7%	90%	8%	91%	11%	81%
ADMA	15%	--	12%	95%	14%	96%	10%	103%
SDMA	-- *	--	23%	114%	11%	104%	6%	94%
total DMA ¹⁾	7%	--	7%	89%	8%	97%	8%	93%
alpha-AAA	-- *	--	15%	67%	5%	64%	10%	57%
Carnosine	-- *	--	7%	88%	6%	101%	11%	98%
Creatinine	1%	--	2%	100%	2%	101%	3%	103%
Histamine	-- *	--	5%	71%	7%	72%	10%	61%
Kynurenine	9%	--	8%	97%	5%	120%	9%	114%
Met-SO	15%	--	5%	101%	6%	107%	12%	98%
Nitro-Tyr ²⁾	-- *	--	5%	78%	7%	82%	6%	66%
c4-OH-Pro	-- *	--	--	--	6%	105%	7%	89%
PEA	-- *	--	3%	83%	3%	91%	3%	84%
Putrescine	-- *	--	5%	92%	2%	100%	4%	102%
Serotonin	10%	--	4%	94%	5%	102%	4%	99%
Spermidine	-- *	--	3%	63%	2%	62%	2%	67%
Spermine	-- *	--	2%	90%	4%	58%	4%	80%
Taurine	4%	--	5%	103%	5%	103%	4%	102%

1) Not analyzed with the UHPLC method

2) Not analyzed with Thermo TSQ Vantage

* detected concentrations below LLOQ

2.3.2 FIA-MS/MS Part of the AbsoluteIDQ® p180kit

The linearity of the FIA part was determined by analyzing 17 different standard chemicals at 14 - 20 different concentration levels. The standards represented 12 acylcarnitines, 4 lipids from each lipid class and one hexose for quantitative analytes.

A linear regression was performed using data of six repetitive preparations in phosphate buffered saline (PBS) plus 4% human serum albumin (HSA) by plotting the mean concentrations of the replicates against the theoretical amount (acylcarnitines were presented in plasma due to their hydrophobicity). R^2 values were calculated, whereby most quantitative analytes exhibited values of $R^2 \geq 0.990$. The following analytes did not meet this criterion:

– C3	($R^2 = 0.969$)
– C6 (C4:1-DC)	($R^2 = 0.987$)
– C10	($R^2 = 0.982$)
– C12	($R^2 = 0.965$)
– C14	($R^2 = 0.912$)
– C16	($R^2 = 0.937$)
– C18	($R^2 = 0.888$)

For each standard, intra-day precision and accuracy were determined at different concentration levels. For the given quantification ranges, the CVs were well below 10% in nearly all cases. In a few cases, the CVs were in the range of 10 - 20%. Regarding accuracy, all quantitative analytes were found in the range of 80 - 115% of the theoretical value.

FIA measurements of glycerophospho- and sphingolipids are based on four different lipid standards, and linearity ranges of those relative-quantitative analytes were not evaluated further.

Two levels of standard mixes were spiked into pooled human plasma and measured in replicates of six. The validation results for precision and accuracy are shown in Table 18 overleaf. The values are based on the SCIEX 4000 QTRAP® and are similar for all other MS platforms. The accuracy was calculated with reference to the actual concentrations of the analytes in the standard mix.

Table 18: Intra-day precision and accuracy of analytes determined for FIA part using human pool plasma

Analyte	Plasma non-spiked		Plasma spiked level 1		Plasma spiked level 2	
MetIDQ short name	CV	Accuracy	CV	Accuracy	CV	Accuracy
C0	3%	--	5%	96%	6%	100%
C10	15%	--	2%	92%	5%	98%
C12	10%	--	8%	103%	4%	109%
C14	-- *	--	9%	97%	4%	109%
C16	8%	--	7%	99%	6%	111%
C18	22%	--	8%	100%	5%	110%
C2	5%	--	6%	101%	4%	109%
C3	10%	--	5%	103%	6%	123%
C4	18%	--	8%	97%	6%	114%
C5	19%	--	8%	99%	8%	112%
C6 (C4:1-DC)	-- *	--	8%	97%	9%	103%
C8	-- *	--	6%	91%	6%	99%
PC aa C24:0	-- *	--	6%	99%	4%	106%
PC aa C36:0	6%	--	8%	95%	4%	99%
lysoPC a C18:0	6%	--	8%	101%	4%	106%
SM C18:0	6%	--	7%	112%	4%	123%
H1	4%	--	6%	98%	3%	102%

* detected concentrations below LLOQ

2.4 Functional sensitivity

A substantial number of the acylcarnitines measured in the FIA part are often below the limit of detection (LOD) in normal plasma. However, since it is well known that metabolite concentrations may change dramatically in disease states or upon external influences, it is important to measure those as well. All LODs listed in Table 3 and Table 6 - Table 8 represent 3x the median background noise of a huge database of independent kit analyses. In MetIDQ™, however, the user can choose to reference to the LOD values that are directly calculated from the three zero samples of the current plate run. This is highly recommended, because the LODs strongly depend on the system condition and should be calculated for each plate run individually. Please note that for many analytes that are measured in the LC part the zero sample measurements often deliver zero as a result when there is no (integrated) peak in the zero samples.

In the FIA part, the quantification limits were experimentally determined in plasma for the quantitative metabolites. In the LC part, the quantification limits correspond to calibration standard level 1 and level 7, respectively. By definition, the LLOQ considers the lowest concentration that meets all quality criteria with respect to accuracy and precision according to guidelines for bio-analytical method validation.

With respect to the upper limit (ULOQ) of analytes measured in FIA mode, the values were set by taking the upper limits of the linearity ranges. In case of relative-quantitative analytes (suitable standards were not available), adequate values could not be determined, and LLOQ and ULOQ are not given. The evaluated quantification ranges of the kit are listed in Table 3 - Table 8.

2.5 Reproducibility and accuracy

The reproducibility of the kit was determined by measuring three concentration levels of human plasma (low, medium, high, all spiked with available standards) in replicates of 6 on three different MS instruments. For acylcarnitines and hexose, the coefficients of variation (CVs) were all below 15%. For glycerophospho- and sphingolipids, the CVs were all below 20% (most below 15%). For amino acids and biogenic amines, the inter-day precision and accuracy was determined at three different levels of spiked human pool plasma. For amino acids, plasma level “low” is diluted plasma (1:2 with PBS, 4% HSA), level “medium” is plasma spiked with Cal2, and level “high” is plasma spiked with Cal3. The validation results are summarized in Table 19 over-leaf. The values are based on the SCIEX 4000 QTRAP® and are similar for all other MS platforms.

For biogenic amines, plasma level “low” is human plasma spiked with Cal2, level “medium” is plasma spiked with Cal3, and level “high” is plasma spiked with Cal5. The results are summarized in Table 20. Also, beta tests in four different labs were performed and confirmed both inter-lab and intra-lab reproducibility.

Table 19: Inter-day precision and accuracy of amino acids in human pool plasma

Analyte	Plasma low		Plasma medium		Plasma high	
MetIDQ short name	CV	Accuracy	CV	Accuracy	CV	Accuracy
Ala	13%	106%	13%	97%	10%	100%
Arg	13%	105%	13%	99%	9%	100%
Asn	17%	99%	8%	97%	13%	98%
Asp	35%	99%	22%	101%	11%	120%
Cit	15%	105%	18%	98%	15%	102%
Gln	9%	111%	9%	100%	11%	98%
Glu	17%	113%	14%	107%	12%	103%
Gly	10%	109%	12%	92%	14%	100%
His	8%	110%	13%	101%	10%	101%
Ile	10%	105%	14%	99%	9%	104%
Leu	11%	103%	10%	87%	6%	107%
Lys	11%	113%	10%	97%	8%	105%
Met	15%	159%	13%	120%	14%	108%
Orn	9%	117%	10%	99%	12%	110%
Phe	12%	109%	12%	101%	12%	100%
Pro	7%	102%	8%	99%	13%	95%
Ser	15%	107%	16%	95%	13%	96%
Thr	13%	105%	12%	97%	8%	94%
Trp	11%	109%	9%	97%	8%	100%
Tyr	5%	104%	8%	99%	11%	99%
Val	12%	109%	12%	99%	9%	95%

Table 20: Inter-day precision and accuracy of biogenic amines in human pool plasma

Analyte	Plasma low		Plasma medium		Plasma high	
MetIDQ short name	CV	Accuracy	CV	Accuracy	CV	Accuracy
Ac-Orn	14%	71%	12%	72%	19%	71%
ADMA	23%	114%	16%	108%	10%	99%
SDMA	37%	144%	22%	106%	11%	99%
total DMA	9%	93%	14%	108%	9%	100%
alpha-AAA	34%	78%	25%	63%	17%	51%
Carnosine	14%	84%	9%	96%	14%	99%
Creatinine	5%	100%	4%	101%	4%	100%
Histamine	22%	76%	13%	69%	10%	64%
Kynurenine	15%	105%	16%	122%	16%	120%
Met-SO	26%	48%	16%	72%	23%	91%
Nitro-Tyr ¹⁾	15%	62%	11%	60%	14%	56%
c4-OH-Pro	18%	90%	14%	92%	10%	88%
PEA	12%	86%	10%	82%	10%	85%
Putrescine	13%	125%	9%	116%	7%	117%
Serotonin	13%	103%	13%	99%	5%	96%
Spermidine	12%	107%	17%	86%	16%	91%
Spermine	12%	73%	20%	92%	26%	139%
Taurine	5%	101%	11%	103%	12%	105%

1) Not analyzed with Thermo TSQ Vantage

2.6 Stability

Kit stability and storage:

biocrates® only guarantees a proper kit performance if the plate is stored unopened in its original plastic bag and away from light. Vial box and kit plate must be stored at -20°C or preferably at -80°C . All other items can be stored at room temperature. Please find the respective expiration dates on the individual items and on the side label of the kit box.

Sample and kit preparation:

The kit must be prepared within one working day. After thawing and vortexing, keep the experimental samples on ice while you load them onto the kit plate. If unexpected problems occur during kit preparation and if the MS measurement cannot be started the same day, please immediately contact support@biocrates.com to discuss a solution.

Autosampler stability at 10°C :

LC plate: 2 days (5 days if sarcosine is not of interest)

FIA plate: 5 days

Storage of ready-to-measure LC and FIA plates:

When the plates are not measured within 2 days, store them in the refrigerator at $+4^{\circ}\text{C}$. Never freeze the plates with the diluted extracts below 0°C !

Sample stability:

Biological samples must be stored throughout at -80°C from collection to usage.

For publications about sample stability, please contact support@biocrates.com.

Ordering and technical support

Sales

E-Mail: sales@biocrates.com

Phone: +43 512 579 823

Technical assistance

Web: biocrates.com/support

E-Mail: support@biocrates.com

For research use only | not for use in diagnostic procedures



biocrates.com



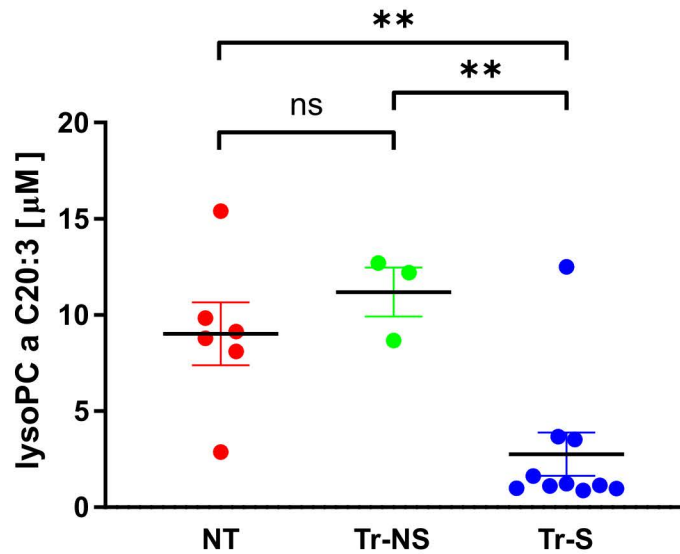
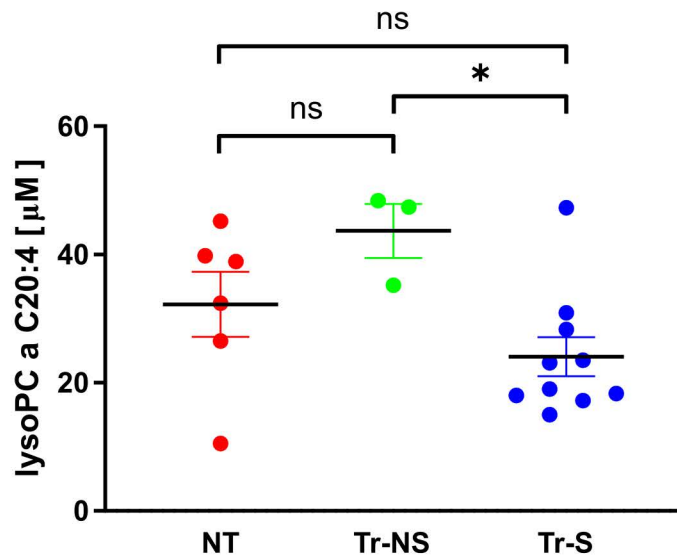
Video Tutorials

www.youtube.com/playlist?list=PLGETE8vMYPlp_gSz4eMaSLG1QKB_mdFpk

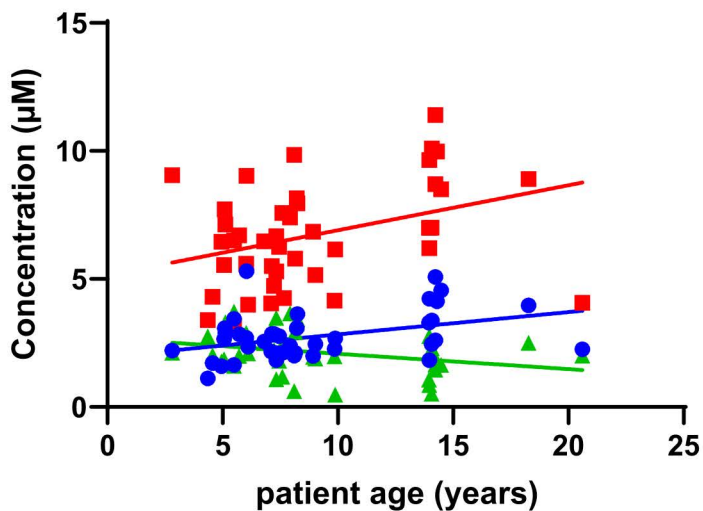


Frequently Asked Questions (FAQ)

<https://support.biocrates.com/tiki-index.php>

(A)**(B)**

(A)



(B)

