Comparability of Absolute*IDQ*® p180 Kit and p400 HR Kit Results

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In metabolic phenotyping endeavors, mass spectrometry is the analytical techniques of choice [1]. To obtain reliable, reproducible results and improve interlaboratory comparability, standardization of the metabolomics workflow is of utmost importance [2]. We enabled comprehensive, accurate, and reproducible assessment of concentrations of various metabolites of all major metabolic pathways through development and validation of two multiparametric metabolomics kits, the Absolute*IDQ*® p180 kit [3,4] and the Absolute*IDQ*® p400 HR kit [5,6,7]. While the Absolute*IDQ*® p180 kit is used for metabolic phenotyping on triple quadrupole (QQQ) mass spectrometers, the Absolute*IDQ*® p400 HR kit was exclusively developed for the high resolution, accurate mass (HRAM) Q Exactive™ family of mass spectrometers based on Orbitrap™ technology (Thermo Fisher Scientific). Although the kits share many characteristics including sample preparation workflow, the obtained results vary due to differences in instrumentation. This application note clarifies the reasons for the discrepancy of results and aids in their interpretation (part I). In addition, results of the analysis of pooled human plasma using the Absolute*IDQ*® p180 kit and the Absolute*IDQ*® p400 HR kit are provided and compared to exemplify parities and disparities of results between to two kits (part II).

Part I

The Kits

The Absolute*IDQ*® p180 kit and p400 HR kit share the same easy-to-use design and enable rapid sample preparation based on a sophisticated 96-well assay format allowing for the use of only minimal sample volumes (10 µL) and high throughput analysis [3-6]. Blank and zero samples, seven calibration standards, and three concentration levels of quality control samples (low, medium, high) are included in each kit. Samples prepared using the Absolute*IDQ*® p180 kit are analyzed by tandem mass spectrometry (MS/MS) in multiple reaction monitoring (MRM) mode on triple quadrupole (QQQ) instruments, whereas samples prepared using the Absolute*IDQ*® p400 HR kit are analyzed in full scan mode on a high resolution, accurate mass (HRAM) mass spectrometer. With the Absolute*IDQ*® p180 kit either high performance liquid chromatography (HPLC) or ultra high performance liquid chromatography (UHPLC) and flow injection analysis (FIA) can be used for chromatographic separation of the sample while for the Absolute*IDQ*® p400 HR kit UHPLC and FIA are used. In both cases, electrospray ionization (ESI) serves as sample inlet method. As part of all our kit products, Biocrates' proprietary, multifunctional Met*IDQ*™ workflow manager software enables easy sample registration, workflow automation, UHPLC- and FIA-MS data processing, technical validation, and statistical analysis.

Table 1 lists the numbers of metabolites within each class measured by the use of the individual kits. While either kit enables the analysis of the same numbers (i.e. the same compounds) of amino acids (21), biogenic amines (21), and monosaccharides (1), the numbers of detected compounds within the various lipid classes are different as well as the individual lipids represented by one compound.

Table 1: Metabolite numbers per class dependent on MS platform

Metabolite class	Numbers of metabolites assessible by use of the respective kit: Absolute*IDQ*® p180 kit	Absolute*IDQ*® p400 HR kit	
Amino acids	21	21	
Biogenic amines	21	21	
Monosaccharides	1	1	
Acylcarnitines	40	55	
Lysophosphatidylcholines	14	24	Analytes which results are affected by the type of kit/instrument used for analysis
Phosphatidylcholines	76	172	
Sphingomyelins	15	31	
Diglycerides		18	Lipid profile
Triglycerides		42	
Ceramides		9	
Cholesteryl esters		14	

Interpretation of Results

A consistent challenge in lipidomics, besides defining the contribution of specific lipids to disease development and manifestation, is the distinction of different isobars and isomers [2, 8].

a) Isobars

Hereafter, isobars are defined as substances within a mass range (Δm) of 0.7 Da for QQQ instruments. According to this definition, the

phospholipids phosphatidylcholine (PC) 18:0/15:0 (fig. 1A) and PC-O 18:0/16:0 (fig. 1B) are isobars (Δm = 0.036 Da, ~ 50 ppm). Using QQQ instruments, their masses cannot be differentiated. In general, analysis of metabolites of a specific class using the Absolute*IDQ*® p180 kit is based on monitoring of joint functionalities: for PCs the phosphocholine headgroup is monitored (fig. 1C). Therefore, PC 18:0/15:0 and PC-O 18:0/16:0 cannot be separated based on their Q3 masses.

Using HRAM instruments (such as Thermo Scientific's Q Exactive™) two distinct compounds can be accurately separated down to Δm ≥ 5 ppm, i.e. the lipids PC 18:0/15:0 and PC-O 18:0/16:0 yield individual signals (fig. 2, signal A and B correspond to the $[M+H]^+$ masses of the lipids in fig. 1, respectively). Hence, there is no need to fragment analytes when using the Absolute*IDQ*® p400 HR kit.

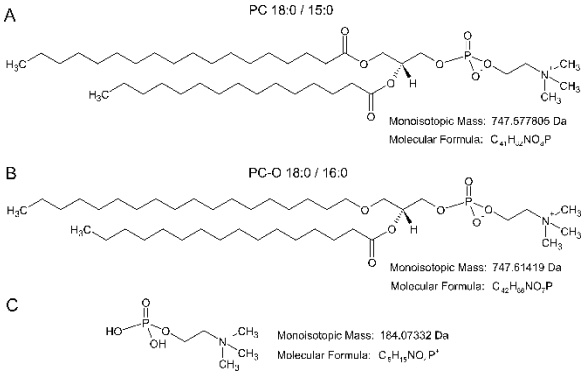

Fig. 1: PC 18:0/15:0 (A) and PC-O 18:0/16:0 (B) are isobars and not distinguishable using the transition of *m/z* 748 → 184 as both lipids share the phosphocholine headgroup with a mass of 184 Da (C).

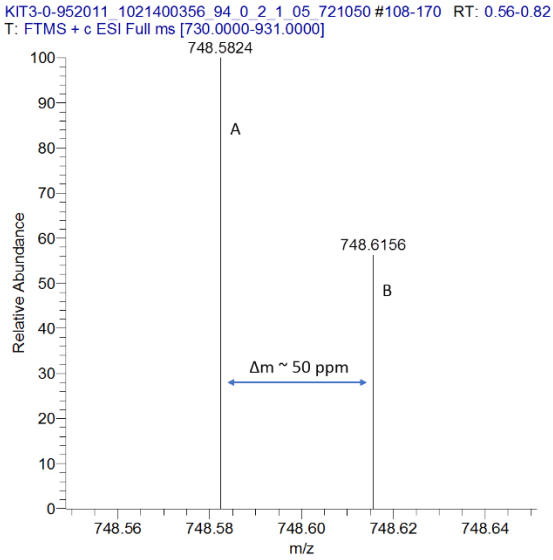

Fig. 2: PC 18:0/15:0 (A) and PC-O 18:0/16:0 (B; $[M+H]^+$ masses) can be distinguished using HRAM instruments with a resolution of 70,000.

b) Isomers

Isomers are compounds of identical molecular formula and therefore identical mass; though of different chemical structures.

Note: Isomers are always isobars.

The lipids PC 18:0/15:0 (fig. 3A) and phosphatidyl ethanolamine (PE) 18:0/18:0 (fig. 3B) are isomers. Therefore, they cannot be distinguished using HRAM mass spectrometry. However, isomers may be distinguished if fragmentation is applied. The Absolute*IDQ*® p180 kit as designed for QQQ instruments uses fragmentation for all metabolite analyses and the two lipids are distinguished by their headgroups (phosphocholine and phosphoethanolamine, respectively).

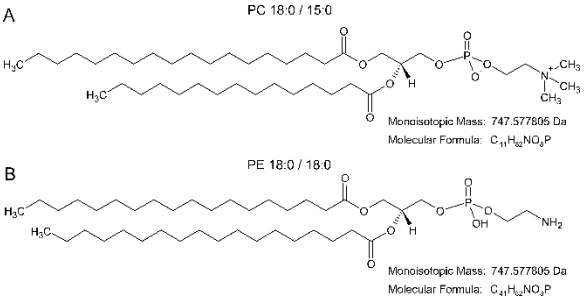

Fig. 3: PC 18:0/15:0 (A) and PE 18:0/18:0 (B) are isomers and cannot be distinguished using HRAM mass spectrometry. If fragmentation is applied using QQQ, they can be distinguished by differences in masses of their headgroups.

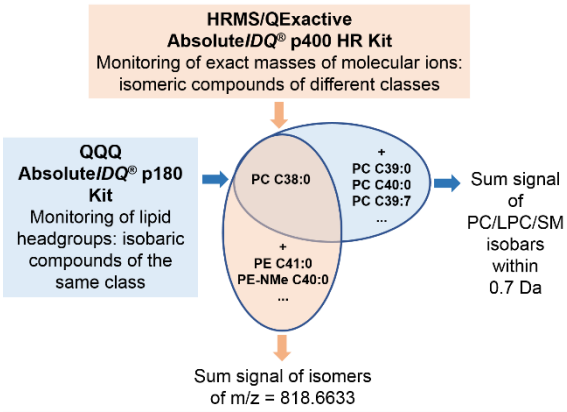

Fig. 4: Comparison of HRAM mass spectrometer/Q Exactive™ and QQQ instrument results exemplified by PC C38:0. HRMS: high resolution, accurate mass-mass spectrometry; LPC: lysophosphatidylcholine; PC: phosphatidylcholine; PE: phosphatidylethanol-

amine; PE-NMe: N-methylphosphatidylethanol-amine; SM: sphingomyelin; QQQ: triple quadrupole mass spectrometer.

Phenylisothiocyanate (PITC) is a reagent for the quantitative derivatization of amino groups. As the exact masses of PEs often correspond to the masses of related PCs (fig. 3, i.e. the compounds are isomers), sum signals of individual lipids are recorded. PITC derivatization is used to separate the signals. Generally, PITC derivatization corresponds to an addition of 135.1877 Da; in the case of PE 18:0/18:0 (m = 747.5778 Da), derivatization shifts the mass to 882.5921 Da, thereby eliminating the PE signal from the respective sum signal. Hence, concentrations of isomeric lipid species can be assessed without interference of PEs. It should be noted that derivatization can be affected by multiple experimental parameters such as the amount of material to derivatize, temperature, or incubation time; therefore, the extent of derivatization may vary slightly between individual experiments. Incomplete derivatization can reflect in variability of results.

Summary

When the Absolute*IDQ*® p180 kit and the Absolute*IDQ*® p400 HR kit are compared, sum signals of isobars within a specific mass range versus sum signals of isomers are analyzed, respectively. Different lipids, often even lipids of different classes (e.g. PCs, lysophosphatidylcholines, and sphingomyelins), underlie the listed sum signal (fig. 4). For guidance as to which lipids are represented by a specific sum signal in accordance with the kits, please refer to the following documents:

Annotation of potential isobaric and isomeric lipid species measured with the Absolute*IDQ*® p180 Kit (and p150 Kit):
https://www.biocrates.com/images/List-of-Isobaric-and-Isomeric-Lipid-Species_v1_2018.pdf

List of Isomers: the Absolute*IDQ*® p400 HR kit – A comparison to the Absolute*IDQ*® p180 kit:
https://www.biocrates.com/images/p400-HR_List-of-Isomers_p180-Comparison_v1_03-2018.pdf

Part II

Analysis of Quality Control Plasma Using the Absolute*IDQ*® p180 and Absolute*IDQ*® p400 HR Kits and Exemplary Comparison of Results

To compare Absolute*IDQ*® p180 and Absolute*IDQ*® p400 HR kit results, Biocrates' quality controls of the highest concentration level (QC3, n = 4; commercially available pooled human plasma spiked with selected analytes for quality control purposes, lyophilized, provided with each kit) were analyzed. Samples were prepared according to the respective user manual. The procedure is identical for both kits.

Materials and Methods

Lyophilized quality control plasma was reconstituted, processed, and measured according to the respective user manual. Specific methods were used for instrumental analysis, which are provided with the kits. A volume of 10 µL of quality controls per well was used. After addition of internal standard and calibration standards, samples were derivatized using PITC. Metabolites were extracted and extracts diluted according to the respective instructions for Absolute*IDQ*® p180 kit analysis using a TSQ Vantage™ (Thermo Scientific) and for Absolute*IDQ*® p400 HR kit analysis using a Q Exactive™ Focus (Thermo Scientific). Analytes measured using UHPLC-MS were quantified using Xcalibur™ (Thermo Scientific), while FIA-MS data analysis was performed using Met*IDQ*™ (Biocrates); for results please see table 2. Outliers were removed and means were calculated when at least two out of four samples showed analyte concentrations above the limit of detection (LOD). Concentrations of sum signals were calculated according to the list of isomers for Absolute*IDQ*® p180 and Absolute*IDQ*® p400 HR kit comparison (List of Isomers: the Absolute*IDQ*® p400 HR kit – A comparison to the Absolute*IDQ*® p180 kit) and as indicated in table 2. Ratios of concentrations assessed using the Absolute*IDQ*® p400 HR kit versus the Absolute*IDQ*® p180 kit were calculated and grouped according to the range of discrepancy for an overview of comparability of results.

Results

In the chosen matrix, concentrations of 186 compounds/sum signals of compounds (hereafter referred to as compounds) were measured and Absolute*IDQ*® p400 HR kit results were compared to those of the Absolute*IDQ*®

p180 kit (table 2). Using the Absolute*IDQ*® p180 kit and the Absolute*IDQ*® p400 HR kit, 32 and 37 compounds were < LOD, respectively. Therefore, a total of 138 ratios of compound concentrations were calculated.
Concentrations of most compounds assessed by the use of the Absolute*IDQ*® p180 kit and the respective compounds assessed using the Absolute*IDQ*® p400 HR kit were within a similar range, i.e. ratios of 59 out of 138 compounds (43%) were between 0.85-1.15, i.e. Absolute*IDQ*® p400 HR kit results were within the range of ± 15% of Absolute*IDQ*® p180 kit results (fig. 5). Within the range of ± 30% fell even 95 compounds (67%). Only 13 out of 138 analytes (9%) showed a > 2-fold change with PC ae C(30:0) versus PC(29:0) + PC-O(30:0) exhibiting the highest fold change (92-fold).

Table 2: Average concentrations of each respective analyte/sum signal of analytes in quality control plasma (commercially available pooled human plasma spiked with selected analytes for quality control purposes, lyophilized, and reconstituted; n=4) including relative standard deviations (RSDs) assessed using the respective kit and ratios (Absolute*IDQ*® p400 HR kit result/Absolute*IDQ*® p180 kit result) of the respective concentrations.

Absolute*IDQ*® p180 kit			Absolute*IDQ*® p400 HR kit			Concentration Absolute*IDQ*® p400 HR kit/ Absolute*IDQ*® p180 kit
Compound(s)	Concentration [µM]	RSD [%]	Compound(s)	Concentration [µM]	RSD [%]	
Ala	1214	2.4	Ala	1117	3.9	0.92
Arg	297	7.6	Arg	324	7.1	1.09
Asn	276	4.2	Asn	271	0.3	0.98
Asp	320	3.7	Asp	318	4.0	1.00
Cit	280	5.0	Cit	301	2.4	1.07
Gln	1255	6.2	Gln	1270	4.9	1.01
Glu	553	10.6	Glu	613	3.6	1.11
Gly	1577	6.6	Gly	1667	5.6	1.06
His	316	6.1	His	327	5.1	1.03
Ile	345	0.4	Ile	328	5.3	0.95
Leu	320	2.9	Leu*	234	7.9	0.73
Lys	595	7.5	Lys	551	2.9	0.93
Met	336	6.5	Met	285	3.1	0.85
Orn	324	10.5	Orn	341	7.6	1.05
Phe	317	5.2	Phe	341	6.3	1.08
Pro	558	4.6	Pro	553	7.0	0.99
Ser	359	0.9	Ser	332	6.1	0.93
Thr	279	1.9	Thr	292	1.9	1.04
Trp	313	0.8	Trp	341	1.4	1.09
Tyr	325	7.1	Tyr	335	5.8	1.03
Val	680	2.9	Val	575	3.3	0.85
Ac-Orn	30.9	5.5	AcOrn	31.0	7.8	1.00
ADMA	16.0	3.2	ADMA	14.6	1.1	0.91
alpha-AAA	57.8	6.0	alpha-AAA	69.5	4.0	1.20
c4-OH-Pro	56.8	4.1	c4-OH-Pro	59.9	1.1	1.06
Carnosine	32.1	3.0	Carnosine	32.4	7.7	1.01
Creatinine	575	3.1	Creatinine	625	1.4	1.09
DOPA	28.7	2.3	DOPA	28.7	1.6	1.00

Dopamine	44.9	3.1	Dopamine	46.1	4.0	1.03
Histamine	59.7	1.7	Histamine	79.2	1.3	1.33
Kynurenine	92.2	5.4	Kynurenine	62.9	7.1	0.68
Met-SO	57.2	8.3	Met-SO	63.9	1.6	1.12
Nitro-Tyr	75.7	0.5	Nitro-Tyr	77.7	1.0	1.03
PEA	6.72	1.7	PEA	10.0	9.3	1.50
Putrescine	6.19	1.1	Putrescine	5.68	1.7	0.92
Sarcosine	58.7	9.7	Sarcosine	56.6	5.0	0.96
SDMA	6.05	7.9	SDMA	6.05	3.3	1.00
Serotonin	5.73	4.6	Serotonin	5.68	3.7	0.99
Spermidine	13.0	1.6	Spermidine	12.5	4.1	0.96
Spermine	12.3	3.0	Spermine	10.3	8.0	0.84
t4-OH-Pro	60.8	0.5	t4-OH-Pro	57.6	0.5	0.95
Taurine	140	13.0	Taurine	141	0.3	1.01
xLeu	–	–	xLeu	563	2.6	–
C0	89.1	0.6	AC(0:0)	103	1.6	1.16
C2	22.9	1.6	AC(2:0)	28.2	2.3	1.23
C3:1	< LOD	–	AC(3:1)	< LOD	–	–
C3	10.7	4.8	AC(3:0)	14.4	7.0	1.34
C4:1	< LOD	–	AC(4:1)	< LOD	–	–
C4	8.26	3.8	AC(4:0)	9.90	1.3	1.20
C3-OH	< LOD	–	AC(3:0-OH)	< LOD	–	–
C5:1	< LOD	–	AC(5:1)	< LOD	–	–
C5	9.00	8.3	AC(5:0)	13.3	2.7	1.48
C3-DC (C4-OH)	< LOD	–	AC(3:0-DC)+AC(4:0-OH)	0.03	23.4	–
C6:1	< LOD	–	AC(6:1)	< LOD	–	–
C6 (C4:1-DC)	4.32	0.8	AC(4:1-DC)+AC(6:0)	4.46	3.0	1.03
C5-OH (C3-DC-M)	< LOD	–	AC(4:0-DC)+AC(5:0-OH)	< LOD	–	–
C5:1-DC	0.02	17.0	AC(5:1-DC)+AC(7:0)	< LOD	–	–
C5-DC (C6-OH)	< LOD	–	AC(5:0-DC)+AC(6:0-OH)	0.19	2.5	–
C8	6.42	8.7	AC(8:0)	7.76	2.1	1.21
C5-M-DC	< LOD	–	AC(6:0-DC)	< LOD	–	–
C9	< LOD	–	AC(8:1-OH)+AC(9:0)	< LOD	–	–
C7-DC	< LOD	–	AC(7:0-DC)	< LOD	–	–
C10:2	< LOD	–	AC(10:2)	0.01	1.4	–
C10:1	< LOD	–	AC(10:1)	0.04	1.9	–
C10	4.65	4.5	AC(10:0)	4.51	3.9	0.97
C12:1	< LOD	–	AC(12:1)	0.04	10.9	–
C12	10.1	4.5	AC(12:0)	10.4	1.5	1.04
C14:2	0.02	14.8	AC(14:2)	0.03	7.1	1.23
C14:1	0.07	3.5	AC(14:1)	0.06	1.1	0.86
C14	4.99	0.9	AC(14:0)	5.75	4.7	1.15
C12-DC	< LOD	–	AC(12:0-DC)	< LOD	–	–
C14:2-OH	< LOD	–	AC(14:2-OH)	< LOD	–	–
C14:1-OH	0.02	5.9	AC(14:1-OH)+AC(15:0)	< LOD	–	–

C16:2	< LOD	–	AC(16:2)	< LOD	–	–
C16:1	< LOD	–	AC(16:1)	0.01	10.1	–
C16	9.53	3.1	AC(14:1-DC)+AC(16:0)	9.09	1.9	0.95
C16:2-OH	< LOD	–	AC(16:2-OH)	< LOD	–	–
C16:1-OH	0.02	1.6	AC(16:1-OH)	< LOD	–	–
C16-OH	< LOD	–	AC(17:0)+AC(16:0-OH)	0.03	9.2	–
C18:2	0.04	9.4	AC(18:2)	0.05	2.9	1.26
C18:1	0.07	9.5	AC(18:1)	0.07	7.1	1.09
C18	4.78	3.4	AC(18:0)	4.82	5.2	1.01
C18:1-OH	< LOD	–	AC(18:1-OH)+AC(19:0)	< LOD	–	–
lysoPC a C14:0	< LOD	–	LPC(14:0)	< LOD	–	–
lysoPC a C16:1	1.36	14.1	LPC(16:1)+LPC-O(17:1)	1.43	5.0	1.05
lysoPC a C16:0	70.4	7.3	LPC(16:0)	85.8	5.0	1.22
lysoPC a C17:0	1.25	9.3	LPC(17:0)+LPC-O(18:0)	1.47	3.4	1.18
lysoPC a C18:2	18.3	9.0	LPC(18:2)	7.06	5.0	0.39
lysoPC a C18:1	11.7	9.9	LPC(18:1)	9.17	5.5	0.78
lysoPC a C18:0	136	11.4	LPC(18:0)	105	2.0	0.77
lysoPC a C20:4	4.28	6.6	LPC(20:4)	5.36	4.8	1.25
lysoPC a C20:3	1.16	10.1	LPC(20:3)	< LOD	–	–
lysoPC a C24:0	< LOD	–	LPC(24:0)	< LOD	–	–
PC aa C24:0	60.6	3.6	PC(24:0)	74.2	1.2	1.23
lysoPC a C26:1	< LOD	–	PC-O(26:1)	< LOD	–	–
lysoPC a C26:0	< LOD	–	PC(25:0)+PC-O(26:0)	0.07	11.3	–
PC aa C26:0	< LOD	–	PC(26:0)	< LOD	–	–
lysoPC a C28:1	< LOD	–	PC(27:1)+PC-O(28:1)	< LOD	–	–
lysoPC a C28:0	< LOD	–	PC(27:0)+PC-O(28:0)	< LOD	–	–
PC aa C28:1	1.07	1.8	PC(28:1)+PC-O(29:0)	< LOD	–	–
PC ae C30:2	< LOD	–	PC(29:2)+PC-O(30:2)	< LOD	–	–
PC ae C30:1	0.11	13.4	PC(29:1)+PC-O(30:1)	0.05	12.3	0.44
PC ae C30:0	0.13	12.2	PC(29:0)+PC-O(30:0)	11.6	10.1	91.98
PC aa C30:0	1.84	3.6	PC(30:0)+PC-O(31:0)	1.43	1.2	0.78
PC ae C32:2	0.26	6.8	PC(31:2)+PC-O(32:2)	0.33	9.0	1.28
PC ae C32:1	1.56	1.2	PC(31:1)+PC-O(32:1)	0.35	27.9	0.22
PC aa C32:3	0.23	5.6	PC(32:3)+PC-O(33:3)	< LOD	–	–
PC aa C32:2	1.28	7.5	PC(32:2)+PC-O(33:2)	1.05	4.2	0.82
PC aa C32:1	5.16	4.5	PC(32:1)+PC-O(33:1)	4.54	0.7	0.88
PC aa C32:0	6.59	5.2	PC(32:0)+PC-O(33:0)	6.90	0.9	1.05
PC ae C34:3	5.73	5.4	PC(33:3)+PC-O(34:3)	7.05	5.9	1.23
PC ae C34:2	5.99	3.6	PC(33:2)+PC-O(34:2)	7.82	3.2	1.31
PC ae C34:1	4.01	4.5	PC(33:1)+PC-O(34:1)	5.10	1.9	1.27
PC ae C34:0	0.72	6.5	PC(33:0)+PC-O(34:0)	0.62	3.0	0.86
PC aa C34:4	0.72	7.6	PC(34:4)+PC-O(35:4)	1.88	7.2	2.60
PC aa C34:3	6.95	2.5	PC(34:3)+PC-O(35:3)	9.49	3.8	1.37
PC aa C34:2	245	3.0	PC(34:2)	303	1.3	1.24
PC aa C34:1	137	3.3	PC(34:1)	124	9.9	0.90

PC ae C36:5	9.20	5.9	PC(35:5)+PC-O(36:5)	11.0	5.0	1.19
PC ae C36:4	12.5	6.4	PC(35:4)+PC-O(36:4)	15.0	3.6	1.21
PC ae C36:3	4.95	3.4	PC(35:3)+PC-O(36:3)	6.78	4.7	1.37
PC ae C36:2	7.53	2.5	PC(35:2)+PC-O(36:2)	8.07	6.1	1.07
PC ae C36:1	4.31	6.4	PC(35:1)+PC-O(36:1)	1.98	6.2	0.46
PC ae C36:0	0.39	12.4	PC-O(37:7)+PC(35:0)+PC-O(36:0)	< LOD	–	–
PC aa C36:6	0.30	6.7	PC(36:6)+PC-O(37:6)	0.07	38.1	0.24
PC aa C36:5	7.30	2.0	PC(36:5)	8.80	3.1	1.21
PC aa C36:4	133	4.3	PC(36:4)	131	5.6	0.99
PC aa C36:3	82.1	3.2	PC(36:3)	92.4	4.5	1.13
PC aa C36:2	154	4.9	PC(36:2)	158	4.4	1.03
PC aa C36:1	24.8	5.8	PC(36:1)	0.09	24.0	0.00
PC aa C36:0	1.31	4.6	PC(37:7)+PC(36:0)	< LOD	–	–
PC ae C38:6	4.50	5.8	PC(37:6)+PC-O(38:6)	4.84	5.2	1.07
PC ae C38:5	12.9	6.6	PC(37:5)+PC-O(38:5)	16.0	3.7	1.24
PC ae C38:4	9.28	5.0	PC(37:4)+PC-O(38:4)	11.1	1.4	1.19
PC ae C38:3	2.88	2.8	PC(37:3)+PC-O(38:3)	0.58	16.9	0.20
PC ae C38:2	1.33	9.5	PC(37:2)+PC-O(38:2)	0.72	6.7	0.54
PC ae C38:1	0.65	3.3	PC(37:1)+PC-O(38:1)	0.40	12.9	0.61
PC ae C38:0	0.93	8.2	PC(38:7)+PC(37:0)+PC-O(38:0)	< LOD	–	–
PC aa C38:6	30.8	3.6	PC(38:6)	25.3	2.6	0.82
PC aa C38:5	34.7	3.6	PC(38:5)	26.8	0.6	0.77
PC aa C38:4	89.3	1.0	PC(38:4)	98.8	1.7	1.11
PC aa C38:3	29.3	2.8	PC(38:3)	4.92	40.7	0.17
PC aa C38:0	1.21	3.8	PC(39:7)+PC-O(40:7)+PC(38:0)	0.76	11.7	0.63
PC ae C40:6	2.45	6.1	PC(39:6)+PC-O(40:6)	3.25	9.9	1.33
PC ae C40:5	2.54	5.2	PC(39:5)+PC-O(40:5)	2.53	8.8	1.00
PC ae C40:4	1.80	1.0	PC(39:4)+PC-O(40:4)	1.48	5.6	0.82
PC ae C40:3	1.00	1.5	PC(39:3)+PC-O(40:3)	0.44	28.1	0.44
PC ae C40:2	0.89	5.7	PC(40:9)+PC(39:2)+PC-O(40:2)	4.92	1.9	5.50
PC ae C40:1	0.79	4.3	PC(40:8)+PC(39:1)+PC-O(40:1)	1.08	36.1	1.37
PC aa C40:6	11.7	4.5	PC(40:6)	19.0	11.7	1.63
PC aa C40:5	7.37	3.7	PC(40:5)	8.60	2.0	1.17
PC aa C40:4	3.41	1.0	PC(40:4)	3.50	11.0	1.02
PC aa C40:3	0.30	4.3	PC(40:3)	< LOD	–	–
PC aa C40:2	0.21	8.9	PC(40:2)	0.65	3.0	3.16
PC aa C40:1	< LOD	–	PC(41:8)+PC(40:1)	0.77	12.6	–
PC ae C42:5	1.23	4.1	PC(41:5)+PC-O(42:5)	1.26	7.3	1.02
PC ae C42:4	0.64	7.7	PC(41:4)+PC-O(42:4)	2.10	8.2	3.28
PC ae C42:3	0.42	6.2	PC(42:10)+PC(41:3)+PC-O(42:3)	1.61	13.7	3.82
PC ae C42:2	0.34	8.1	PC(41:2)+PC-O(42:2)	< LOD	–	–
PC ae C42:1	0.21	5.8	PC(41:1)+PC-O(42:1)	< LOD	–	–
PC ae C42:0	< LOD	–	PC(42:7)+PC-O(42:0)	0.11	18.4	–

PC aa C42:6	0.29	7.7	PC(42:6)	0.19	8.2	0.67
PC aa C42:5	0.18	13.6	PC(42:5)	0.06	14.2	0.30
PC aa C42:4	0.14	6.9	PC(42:4)	0.68	3.3	4.97
PC aa C42:2	0.17	12.6	PC(42:2)	2.51	31.2	14.92
PC aa C42:1	0.18	2.0	PC(42:1)	< LOD	–	–
PC aa C42:0	0.33	12.0	PC(42:0)	< LOD	–	–
PC ae C44:6	0.87	0.7	PC(43:6)+PC-O(44:6)	1.53	11.3	1.76
PC ae C44:5	0.86	7.5	PC(44:12)+PC-O(44:5)	0.41	10.4	0.48
PC ae C44:4	0.24	12.3	PC-O(44:4)	< LOD	–	–
PC ae C44:3	0.06	26.2	PC(44:10)+PC-O(44:3)	< LOD	–	–
SM (OH) C14:1	2.73	4.8	SM(33:1)	17.4	9.9	6.37
SM C16:1	10.1	5.3	SM(34:2)	11.6	1.1	1.16
SM C16:0	75.6	5.6	SM(34:1)	85.4	1.2	1.13
SM (OH) C16:1	1.98	6.9	SM(35:1)	2.04	4.7	1.03
SM C18:1	9.69	4.8	SM(36:2)	5.78	4.2	0.60
SM C18:0	22.3	4.8	SM(36:1)	13.8	5.0	0.62
SM C20:2	0.10	4.7	SM(38:3)	0.58	24.7	6.01
SM (OH) C22:2	6.60	4.6	SM(41:2)	7.47	6.6	1.13
SM (OH) C22:1	8.47	6.0	SM(41:1)	10.6	1.1	1.25
SM C24:1	40.0	3.1	SM(42:2)	49.4	5.1	1.23
SM C24:0	18.6	9.2	SM(42:1)	21.6	2.6	1.16
SM (OH) C24:1	0.75	10.3	SM(43:1)	1.50	7.6	1.99
SM C26:1	0.17	15.5	SM(44:2)	0.65	5.0	3.91
SM C26:0	0.05	34.9	SM(44:1)	0.45	6.7	8.96
H1	21178	4.2	H1	20786	4.5	0.98

* The concentration of leucine was calculated from the concentrations of xLeu (leucine + isoleucine) and isoleucine.

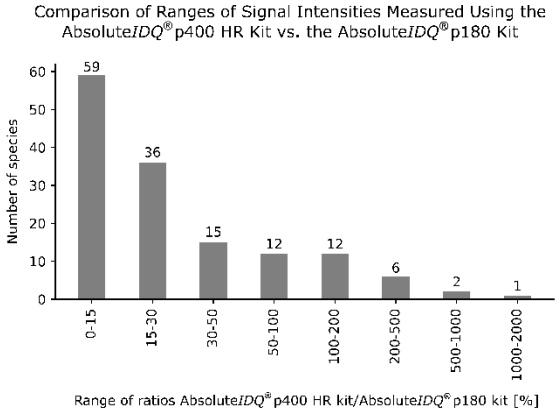

Fig. 5: Numbers of compounds within the respective ranges of their Absolute*IDQ*® p400 HR kit/Absolute*IDQ*® p180 kit ratio.

Discussion

Our data show that for most compounds concentrations in pooled plasma samples assessed using the Absolute*IDQ*® p180 kit and Absolute*IDQ*® p400 HR kit are comparable, i.e. the deviation of results between kits is within 30% for the majority of compounds. Variance in concentrations for certain signals between the kits can be due to the analytical peculiarities of the method of analysis as explained in part I of this application note. Whether using the Absolute*IDQ*® p180 kit or the Absolute*IDQ*® p400 HR kit yields higher concentrations in a particular matrix, is dependent on which signals contribute to the respective sum signal. As dilutions for instrumental analysis were prepared from the same extract, differences in results between the two kits due to variance in sample preparation such as derivatization efficiency can widely be ruled out. It is particularly important to keep in mind that different individual signals underlie one sum signal dependent on which kit/instrument type is used for analysis. It should also be noted that this comparison only represents signal ratios under the distinct experimental conditions. In case of other matrices or even the same matrix

from a different subject pool, metabolite concentrations and ratios will likely be different. As the lipid species underlying the individual signals can vary widely in their concentrations, a range from matrix to matrix and even from sample to sample within one matrix is to be expected. In case of a comparison of Absolute*IDQ*® p180 kit and Absolute*IDQ*® p400 HR kit data, it is advisable to examine the data on a case to case basis.

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