Analytica Chimica Acta 1137 (2020) 74–84

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Determination of one year stability of lipid plasma profile and comparison of blood collection tubes using UHPSFC/MS and HILIC-UHPLC/MS

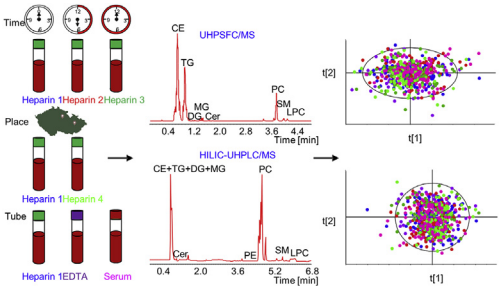
Denise Wolrab [a, *], Michaela Chocholoušková [a], Robert Jirásko [a], Ondřej Peterka [a], Vladimíra Mužáková [b], Hana Študentová [c], Bohuslav Melichar [c], Michal Holčapek [a]

[a] University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10, Pardubice, Czech Republic
[b] University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences, Studentská 573, 532 10, Pardubice, Czech Republic
[c] Department of Oncology, Faculty of Medicine and Dentistry, Palacký University and University Hospital, I.P. Pavlova 6, 775 20, Olomouc, Czech Republic

HIGHLIGHTS

- Quality control system is developed for the quality assurance of lipidomic quantitation.
- Lipidomic profiles are less influenced by the type of blood collection tubes than by the effect of time and collection site.
- Method dependent measurement bias can be compensated by the reference value normalization.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 May 2020
Received in revised form
24 August 2020
Accepted 29 August 2020
Available online 6 September 2020

Keywords:
Lipids
Lipidomics
Supercritical fluid chromatography
Liquid chromatography
Mass spectrometry
Human plasma
Serum
Blood collection

ABSTRACT

Effects of blood collection tubes, the time period, the sample origin, and the method used on the lipidomic profile are investigated by ultrahigh-performance supercritical fluid chromatography - mass spectrometry (UHPSFC/MS) and hydrophilic interaction liquid chromatography ultrahigh-performance liquid chromatography - mass spectrometry (HILIC-UHPLC/MS). Heparin plasma samples have been obtained from 99 healthy volunteers at three time points separated by six-month intervals together with one collection for EDTA plasma and serum. Furthermore, lipid concentrations in heparin plasma collected at two different sites are compared. 171 lipid species from eight lipid classes are quantified with UHPSFC/MS, and 122 lipid species from four lipid classes with HILIC-UHPLC/MS. The accuracy of both methods is monitored by the quantitation error using two internal standards (IS) per individual lipid classes. No significant differences in lipid profiles are observed for different time points and types of collection tubes (heparin plasma, EDTA plasma, and serum). Most pronounced lipid concentration differences are observed for the comparison of NIST SRM 1950 human plasma and mean lipid concentrations of the investigated cohort. Furthermore, differences in lipid concentrations are observed between employed methods (UHPSFC/MS *vs.* HILIC-UHPLC/MS), which can be compensated by the normalization using NIST SRM 1950 human plasma used as the quality control sample.

* Corresponding author.
E-mail address: denise.wolrab@upce.cz (D. Wolrab).

https://doi.org/10.1016/j.aca.2020.08.061
0003-2670/

1. Introduction

The analysis of blood samples is commonly performed for various medical examinations and for research purposes. The collection of venous blood is a routine medical procedure [1], easily feasible without the need of special equipment, and a minimal invasive intervention for humans. Different sampling tubes are available with and without additives, whereby additives should prevent or initiate a blood clotting. If the blood is coagulated to form the blood clot, then the supernatant obtained after the centrifugation is called serum. On the other hand, the presence of anticoagulants prevents the blood clotting, and plasma is the supernatant after centrifugation of non-coagulated blood. Therefore, the main difference between plasma and serum is that plasma contains fibrinogen, a clotting factor, in comparison to serum. Plasma can be directly processed after the blood sampling, whereby the blood sample has to be kept for some time to ensure complete clotting in case of serum. The detailed investigation of differences between plasma and serum on the metabolomic level is of interest, because the choice of sample material may affect the outcome of research studies [2–9], especially those searching for metabolomic differences depending on the pathological state [10,11]. The metabolomic changes over time is another parameter that may be of interest for clinical studies. In general, the determination of the extent of metabolomic profile changes coming from preanalytical and exogenous factors like the sample collection procedure, the time period, measurement bias, and biological variance is essential for the evaluation of characteristic changes coming from endogenous factors including the health state [10,12–14].

Lipidomics is a subgroup of metabolomics dealing with the identification and quantitation of lipids. Lipids are biomolecules involved in various biological processes, such as energy storage, signaling, and building blocks for cell membranes. Recently, lipid concentration and composition changes were associated with several diseases, such as cancer [15]. Therefore, lipidomics gains more attention in clinical research studies [16]. Lipids are commonly analyzed using MS as a standalone method or coupled to chromatography [17], where the lipid species or the lipid classes are separated depending on the chromatographic mode used. Lipid class separation combined with high-resolution mass spectrometry, has the advantage that one IS per lipid class can be used for the quantitation. The lipid class specific internal standard is co-eluting with other lipid species belonging to the same lipid class, leading to comparable matrix effects. The ionization efficiency depends on the structure of the lipid species, whereby the head group of lipids have a higher impact on the ionization than the fatty acyl chains. The different response factors depending on the hydrophobic part of the lipid species may lead to a small systematic quantitation error using 1 internal standard per lipid class. However, as all samples are affected by the same concentration shift, the outcome of comparison studies, i.e. biomarker discovery studies is not influenced. Therefore, the use of one IS per lipid class for lipid quantitation using lipid class separation approaches or shotgun/MS is so far the best quantitation approach and widely accepted in the lipidomics community. The class separation is achieved by the interaction of the head group of lipids with the polar stationary phase and the elution with solvents with higher elution strength diminishing the interaction. Chromatographic techniques leading to lipid class separation are HILIC-UHPLC/MS [17–19] and UHPSFC/MS [20].

The aim of the present study is the investigation of the influence of blood collection tubes, the sample collection site, and the sample origin on the lipidomic profile as well as time dependent changes measured by UHPSFC/MS and HILIC-UHPLC/MS.

2. Experimental section

2.1. Reagents and chemicals

The following solvents and additives for the extraction and chromatographic analyses were of LC-MS grade (Chromasolv-Honeywell, Riedel-de Haën, Germany) and purchased from Sigma Aldrich (St. Louis, MI, USA) or Fisher Scientific (Waltham, Massachusetts, USA): methanol (MeOH), acetonitrile (ACN), water (ultra H_2O), 2-propanol (IPA), hexane, ammonium carbonate, and ammonium acetate (NH_4OAc). Lichrosolv chloroform ($CHCl_3$) for the extraction was purchased from Merck (Darmstadt, Germany). Water was prepared by the in-house Milli-Q purification system (Millipore, Molsheim, France) from distilled water and used for the preparation of mobile phases for HILIC-UHPLC/MS analysis and the preparation of the ammonium carbonate buffer for the extraction. Supercritical carbon dioxide ($scCO_2$) with 99.995% purity was purchased from Messer (Bad Soden, Germany). The following lipids used as IS or system suitability standard (SSS) were purchased either from Avanti Polar Lipids (Alabaster, AL, USA), Nu-Chek (Elysian, MN, USA), or Merck: cholesteryl-d_7 palmitate, *N*-lauroyl-D-*erythro*-sphingosine, *N*-heptadecanoyl-D-*erythro*-sphingosine, *N*-stearoyl-D-*erythro*-sphingosine (d_7), didocenoin (DG 12:1(11Z)/0:0/12:1(11Z)), 3-di-(9z-octadecenoyl)-2-hydroxy-*sn*-glycerol-d_5, 1-pentadecanoyl-2-oleyol(d_7)-*sn*-glycerol, 1-heptadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, 1-oleoyl(d_7)-2-hydroxy-*sn*-glycero-3-phosphocholine, monononadecenoin (MG 19:1(10Z)/0:0/0:0), 1-oleoyl(d_7)-*rac*-glycerol, 1-pentadecanoyl-2-oleoyl(d_7)-*sn*-glycero-3-phosphocholine, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine, 1,2-dierucoyl-*sn*-glycero-3-phosphocholine, *N*-lauroyl-D-*erythro*-sphingosylphosphorylcholine, *N*-oleoyl-D-*erythro*-sphingosylphosphorylcholine-d_9, 1,3-dipentadecanoyl-2-oleyol(d_7)-glycerol, trinonadecenoin, monoolein, diolein, triolein, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine, 1,2-dioleoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt), *N*-oleoyl-D-*erythro*-sphingosylphosphorylcholine, *N*-oleoyl-D-*erythro*-sphingosine, D-*erythro*-sphingosine, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt), D-lactosyl-ß-1,1′ *N*-palmitoyl-D-*erythro*-sphingosine, D-galactosyl-ß-1,1′ *N*-lauroyl-D-*erythro*-sphingosine, and D-glucosyl-ß-1,1′-*N*-palmitoyl-D-*erythro*-sphingosine. The IS mixture as well as the SSS mixture were prepared and diluted as published previously [20].

2.2. Biological samples

All donors signed the informed consent for the inclusion in this study. The following types of blood collection tubes were used for the isolation of plasma, heparin (Vacuette® Tube 9 mL LH Lithium Heparin 16 × 100 green cap-black ring, non-ridged) and ethylenediaminetetraacetic acid (EDTA) (Vacuette® Tube 9 mL K3E K3EDTA 16 × 100 lavender cap-black ring, non-ridged), and for the isolation of serum (Vacuette® Tube 9 mL CAT Serum Clot Activator 16 × 100 red cap-black ring, non-ridged). Blood samples were collected from the same volunteers of 57 females and 42 males in 6-month intervals. At the first collection, heparin-lithium plasma (in the following text abbreviated as heparin 1), at the second collection heparin plasma and serum (heparin 2 and serum), and at the third collection heparin and EDTA plasma (heparin 3 and EDTA) were isolated. This sample set allows the evaluation of the influence of the sample collection tubes on the lipidome as well as the evaluation of lipidomic changes over time. Furthermore, heparin plasma samples from another 32 females and 49 males (heparin 4) were obtained from a different blood collection place for the

evaluation of differences in lipidomic profiles between different collection sites and subjects.

2.3. Sample preparation

Blood collection tubes were kept for 30 min at room temperature after the blood drawing. EDTA plasma and heparin plasma were isolated by the centrifugation at 2500 rpm, 20 °C for 10 min, and then 2 mL of the supernatant was transferred to Eppendorf tubes. The serum was isolated by the centrifugation at 3000 rpm, 20 °C for 15 min, and then 2 mL of the supernatant were transferred to Eppendorf tubes. Eppendorf tubes were stored at −80 °C until further processing for the lipidomic analysis.

2.4. Quality control

A pooled sample of heparin plasma and serum samples was prepared and further used as quality control (QC) sample together with the NIST Standard reference material 1950 human plasma (further abbreviated as NIST plasma). A mixture of natural occurring lipid standards containing 18:1 fatty acyls was used as SSS [20]. The order of the samples was randomized for extractions performed within 6 working days to avoid biases, which could be caused by extraction blocks of the same sample type. Extractions of the QC sample (every day) and the NIST plasma (every other day) were performed to monitor the extraction process. Mixtures of QC extracts from all extraction days (QC sample) and each extraction day (six batch quality control samples - BQC) were prepared. For MS measurements, samples were again randomized using the Microsoft Excel Kutools Add-In. The sample block of blank, QC, and SSS was measured after every 40 samples. Injections of blanks, calibration curve, NIST plasma, QC, SSS, and BQC were measured at the beginning, in the middle, and at the end of the whole sequence.

2.5. Sample extraction protocol

Liquid-liquid extraction was used for the sample preparation [20]. In brief, the IS mixture (20 μL), the human blood sample (25 μL plasma or serum), $CHCl_3$ (2 mL), and MeOH (1 mL) were placed in 4 mL glass vials and homogenized in an ultrasonic bath (15 min, 30 °C). When samples reached ambient temperature, 600 μL ammonium carbonate buffer (250 mM) was added, samples were ultrasonicated (15 min, 30 °C), centrifuged (867×*g*, 3000 rpm, 3 min), and the organic layer was transferred into 8 mL glass vials. The extraction was repeated by adding 2 mL $CHCl_3$ to the aqueous phase, followed by ultrasonication (15 min, 30 °C), centrifugation (3000 rpm, 3 min), and combining both organic phases. The organic phase was evaporated under a gentle stream of N_2, and dried extracts were stored at −80 °C. On the day of measurement, when samples reached ambient temperature, extract residues were dissolved in 500 μL $CHCl_3$/MeOH (1/1, *v*/*v*), vortexed for 10 s, and transferred into HPLC vials for measurements. The samples were diluted 1:5 (*v*/*v*) with $CHCl_3$/MeOH (1/1, *v*/*v*) before UHPSFC/MS and HILIC-UHPLC/MS analyses.

2.6. UHPSFC/MS

The UHPSFC/MS method was the same as previously published [20,21]. An Acquity UPC^2 (Waters, Milford, MA, USA) was hyphenated to a Synapt G2-Si mass spectrometer (Waters) using the commercial interface for UHPSFC/MS coupling (Waters). For the lipid class separation, the Viridis BEH column (100 × 3 mm, 1.7 μm) was used. The mobile phase A was $scCO_2$, and MeOH with 1% ultra H_2O and 30 mM of NH_4OAc was used as the mobile phase B (also called modifier) and the make-up solvent. The following gradient was used including the reequilibration: 0 min−1% B, 1.5 min−16% B, 4 min−51% B, 7 min−51% B, 7.51 min−1% B, and 8 min−1% B. The column temperature was 60 °C, the automatic back-pressure regulator was set to 1800 psi, the flow rate to 1.9 mL min^{-1}, the injection volume to 1 μL, and the make-up flow rate to 0.25 mL min^{-1}. Electrospray ionization in positive ion mode was used, and full scan mass spectra were measured in the mass range of *m/z* 150–1200 using the sensitivity mode. The continuum mode with the scan rate of 0.5 s was used for the analysis, and the lock mass leucine enkephalin, recorded each 15 s at a flow rate of 20 μL min^{-1}, was used for offline mass correction.

2.7. HILIC-UHPLC/MS

The HILIC-UHPLC/MS method was the same as previously published [20]. An Agilent 1290 Infinity UHPLC (Agilent Technologies, Waldbronn, Germany) was hyphenated to a Xevo G2-XS-QTOF mass spectrometer (Waters, Milford, MA, USA). For the lipid class separation, the Viridis BEH column (100 × 3 mm, 1.7 μm) was used. The mobile phase A was ACN/H_2O (96/4, *v*/*v*), the mobile phase B was ACN/H_2O (2/98, *v*/*v*), and both phases contained 8 mM of NH_4OAc. The following linear gradient was used including the reequilibration: 0 min−100% mobile phase A, 5 min−84% of A, 5.5 min−84% of A, 5.51 min−100% A, and 10.5 min−100% A. The column temperature was 40 °C, the flow rate was 0.5 mL min^{-1}, and the injection volume was 1 μL. Electrospray ionization in positive ion mode was used, and full scan mass spectra were measured in the mass range of *m/z* 150–1200 using the sensitivity mode. The continuum mode with the scan rate of 0.5 s was used for the analysis, and the lock mass leucine enkephalin, measured every 15 s at the flow rate of 10 μL min^{-1}, was used for offline mass correction.

2.8. Data analysis

The raw data file size was reduced by applying the noise reduction using the Waters Compression Tool [20]. The Accurate Mass Measure tool from MassLynx was used to apply the lock mass correction for better mass accuracy and to convert the continuum data to centroid data. Retention time windows for each lipid class were evaluated by the comparison of chromatograms of QC samples obtained at the beginning, in the middle, and at the end of the sequence. Methods for each lipid class were generated by setting the determined retention time window for each lipid class and the intensity threshold of 3000 using the MarkerLynx tool from the MassLynx software (Waters). These methods were applied for all samples within the sequence, resulting in a summary table of all *m/z* with the corresponding intensity in each sample. The summary table for each lipid class was exported as. txt file and further processed with the LipidQuant script in Microsoft Excel. The embedded database of lipids in LipidQuant including the exact masses was created based on previously identified lipids in human biological samples using tandem mass spectrometry. The lipid identification was achieved by the use of lipid class internal standards, allowing the assignment of the lipid class and lipid species retention time and employing high-resolution MS for lipid species identification. Summary tables were filtered by the comparison of measured *m/z* with exact masses of lipids included in the database with a predefined tolerance mass window (5 mDa) for the lipid identification. Identified lipids including IS in each sample were quantified by dividing the intensity of the lipid by the intensity of the IS and multiplied with the known concentration of IS. Calculated concentrations were then isotopically corrected, and final concentrations for each lipid species in all samples were automatically summarized in result tables for the data from UHPSFC/MS

and HILIC-UHPLC/MS (Table S-1 and Table S-2). Microsoft Excel was used for the calculation of mean lipid concentrations, the relative standard deviation (RSD), and the normalization. The p-value was calculated by T. TEST function associated to the Student's T-test for two tailed distribution and two sample equal variance T-test.

3. Results and discussion

3.1. General remarks

The blood drawing procedure as well as the type of blood collection tubes may influence the sample quality, integrity, and analyte concentrations. Therefore, recommended guidelines should be followed during blood collection and plasma/serum isolation, *i.e.*, the collection speed, the filling of collection tubes, and the isolation time. In general, it is recommended to use the same type of blood collection tubes for the whole study. However, it may happen that samples are received originating from various types of tubes, for example for multi-center retrospective studies of rare diseases. Third parties (biobanks or clinics) often provide plasma or serum samples for analytical measurements without a simple way to control the quality of provided samples. The influence of the time period by three blood collections during one year and the type of blood collection tubes on the lipidomic profile for the same volunteers is investigated in this study (Fig. S-1) to better understand possible biases in such bioanalytical studies.

3.2. Quality control system

The development of strategies to monitor and evaluate the quality of the sample preparation protocol and the analytical measurement is of special importance for the complex lipidomic analysis of human samples. These strategies should include automated data processing, be practicable and representative for all investigated lipid species within all samples. The manual data inspection and processing of over one hundred lipid species in hundreds of samples would be very laborious, and the introduction of processing errors is likely due to the enormous complexity. For non-automated sample preparation, the special emphasis should be put to keep individual steps for all samples as constant as possible. For MS based methods, a certain loss of sensitivity is unavoidable during the analysis of hundreds of samples with complex matrix due to the contamination of the mass spectrometer over time. The best compensation of quantitation errors due to signal drop is the use of IS eluted and ionized at the same time as the target analyte. The use of isotopically labeled analouges for the quantitation of the target analytes as IS with known concentration would lead to the most accurate quantitation. According to the recommendation of the Lipidomics Standard Initative (LSI) [17,22], lipid class separation approaches hyphenated to high resolution mass spectrometry, like UHPSFC/MS and HILIC-UHPLC/MS, use at least one IS per lipid class under the assumption that ionization effects are the same for the lipid class IS and all analytes from the same class. However, the signal response is structure dependent with the highest impact of the hydrophilic part but also a partial contribution of the hydrophobic part, which results in different response factors. The stable isotope labeled analogues of each lipid species in human plasma are not commercially available. Therefore, the compromise of using isotopically labeled or exogenous IS for lipid class quantitation is widely accepted even though a certain systematic quantitation error is introduced, but this error is supposed to be constant.

Two IS per lipid class were used in this study, which allows to estimate quantitation errors for each lipid class and to monitor the variance of the analytical method. Sample extracts were randomized during measurements in order to avoid that samples belonging to one sample type are measured in series, which may lead to inconclusive results due to measurement artefacts. The SSS, solvent blanks and QC samples, were measured regularly after each 40 samples during the sequence. The signal response of all IS and of lipids containing oleoyl fatty acyls present in the SSS, but also naturally occurring in the samples, were monitored for all samples during sequence measurements, allowing the assessment of the quality of the sample preparation and the instrument performance. For the instrumental performance the SSS and QC samples and for the sample preparation and instrumental performance real samples are evaluated. It is assumed that the selected lipid species behave similarly to all other lipid species belonging to the same lipid class. Fig. 1 illustrates the signal decrease during measurements for each sample monitoring the IS PC 33:1 d_7 (Fig. 1a), the exogenous IS PC 28:0 (Fig. 1b), and the natural occurring PC 36:2 (Fig. 1c). The signal deviations of IS are much less pronounced than for the natural occurring PC 36:2, which confirms a good quality of sample preparation. The retention time shift of less than 6 s for PC 33:1 d_7 for the whole sequence is illustrated in Fig. 1d (step drops are caused by a minimum recordable step 0.01 min for time). The signal drop is compensated, when concentrations of individual samples are calculated (Fig. 1e and f) by the division of the intensity of the lipid species with the intensity of IS and multiplied by the known concentration of the IS. The known concentration of the second IS is used for the calculation of the quantitation error (Fig. 1h), which is lower than 20% for the majority of lipid classes except for ceramides. The clustering of the QC sample in comparison to real samples is illustrated in the principal component analysis (PCA) (Fig. 1g). Fig. 1i shows the number of quantified lipid species in human plasma or serum within 8 investigated lipid classes using UHPSFC/MS. Figure S-2 illustrates the RSD of the IS for all samples investigated in the study employing UHPSFC/MS. An RSD <10% for all IS is observed, except for DG 24:2 (11%) and PC 44:0 (19%). This indicates that the analytical method together with the sample preparation was highly repeatable. The higher RSD of PC 44:0 may be caused by interferences and suggests that PC 44:0 should not be used as IS. It has to be noted that the analytical method will not distinguish differences in the lipidome caused by the collection tube, the sample collection site and the time dependency for the three blood collections during one year, which are smaller than the method error for individual lipid classes, estimated by the RSD for IS (Figure S-2).

3.3. Comparison of the lipidomic profile of three blood collections during one year period

Plasma was collected from the same 99 volunteers in 6-month intervals using heparin blood collection tubes. The blood was always collected in the morning after overnight fasting. It is assumed that the overall lifestyle of individual volunteers, like the food habits and sport activities, have not changed significantly during the timeframe of one year. The mean concentration of each lipid for all volunteers per time point was calculated and compared to each other for UHPSFC/MS (Fig. 2a and Fig. 2b) and HILIC-UHPLC/MS (Fig. 2g and h). Correlation plots of the mean lipid concentration for all volunteers show no significant differences. The calculated p-values are occasionally <0.05 for the various lipid species between individual collections of heparin plasma (Table S-9 and Table S-10), but no statistical significant trends are observed. P-values <0.05 are mainly observed for the comparison of PC between heparin 2 and 3 using HILIC-UHPLC/MS. The intra-individual RSD of the concentrations for all time points for each volunteer and lipid was calculated to elaborate differences of lipid concentration over time for UHPSFC/MS and HILIC-UHPLC/MS (Table S-3 and Table S-6). The

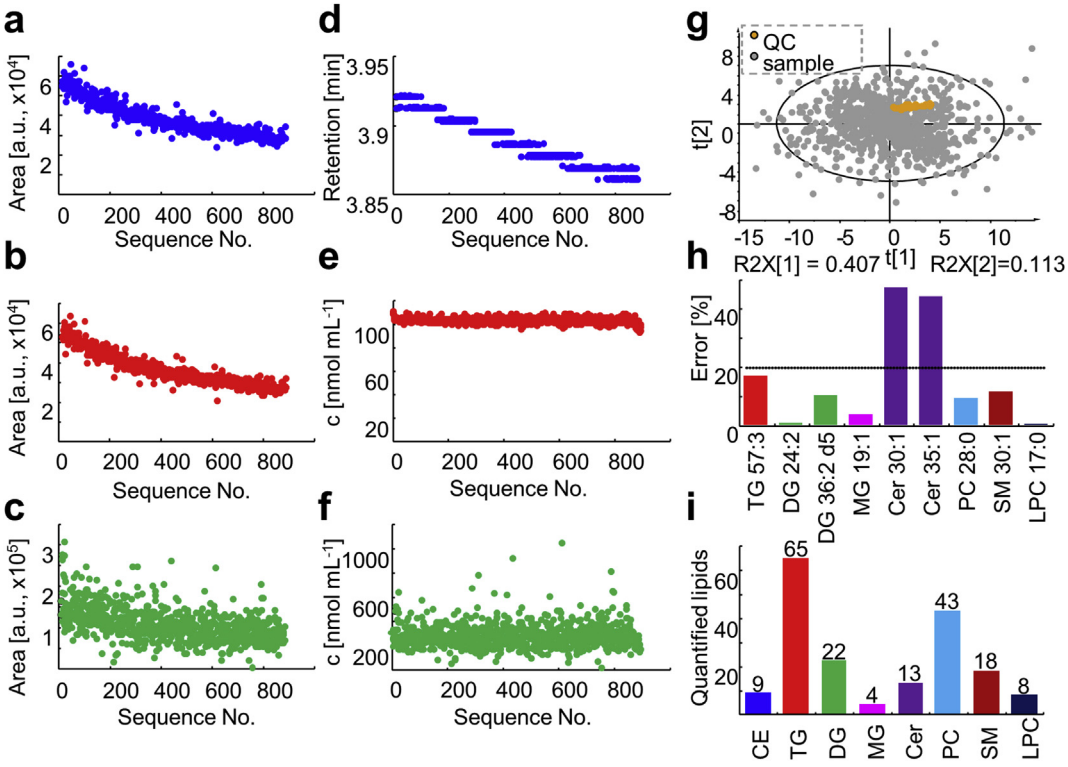


Fig. 1. Quality control of lipidomic analysis using online monitoring of the signal response during UHPSFC/MS measurements for IS in each sample: a) PC 33:1; b) PC 28:0; c) endogenous PC 36:2; d) the retention time shift of PC 33:1 d_7 during measurements (IS). The compensation of the signal drop during measurements by using PC 33:1 d_7 for the quantitation of: e) exogenous PC 28:0; and f) endogenous PC 36:2; g) PCA for all investigated samples with highlighted QC samples for illustrating the clustering; h) calculated quantitation errors by employing multiple IS/lipid class-deuterated IS were used for quantitation of the other IS; i) investigated lipid classes and number of lipid species using UHPSFC/MS.

percentage of the intra-individual RSD <20 and < 50% for individual lipid classes for all volunteers was calculated (Table S-3 and Table S-6). Results indicate that the concentrations of SM, LPC and Cer are relative stable over the time period of one year, whereby MG, DG and TG are prone to concentration changes over time (Figure S-6 and Figure S-7). The intra-individual RSD of the concentrations is <10% for the additional added IS, which were also used for the calculation of the quantitation error. This shows that the sample preparation and measurement error is kept within reasonable limits. Reasons for the biologicaly variability, can be versatile, as exogenous and endogenous factors can alter the lipidome, like the lifestyle, nutrition habits and the health condition. No general trend is observed for lipid concentration changes over the time depending on the gender (Table S-3 and Table S-6). HILIC-UHPLC/MS results for the RSD of different time points for each volunteer show a similar trend, the deviation is higher for non-polar lipids than polar lipid species, with the exception of ether-phosphatidylcholines and short fatty acyl phosphatidylcholines present at relatively low concentrations. Generally, HILIC-UHPLC/MS is more prone to the sensitivity loss during measurements in comparison to UHPSFC/MS, which is probably attributed to faster contamination of the system caused by higher amounts of lipids introduced to the mass spectrometer. The sensitivity loss during measurements using HILIC-UHPLC/MS may lead to the higher RSD values for the low abundant ether-phosphatidylcholines and short fatty acyl phosphatidylcholines, as this trend was not observed with UHPSFC/MS.

3.4. Comparison of EDTA and heparin plasma

The use of EDTA plasma is recommended for metabolomics studies. However, the NIST plasma is a heparin plasma sample and frequently used for QC, method development, method validation, and inter-laboratory comparison studies. Therefore, the systematic study for the comparison of lipidomic profiles obtained for EDTA plasma and heparin plasma for the same volunteers is performed here. At the 3rd collection of heparin samples (heparin 3), EDTA plasma was also collected for 99 volunteers. The mean lipid concentration of 99 volunteers for each lipid species was calculated for EDTA plasma and heparin 3 plasma. Correlation plots for EDTA and heparin 3 plasma show high similarity of lipid concentrations in both matrices (Fig. 2d and j). The RSD of EDTA and heparin 3 for each lipid species and volunteer was calculated (Table S-4 and Table S-7). The RSD was <20% for 81% of all lipids in all volunteers using UHPSFC/MS or 77% for HILIC-UHPLC/MS. Generally, the elevated RSDs were observed for glycerolipids (long-chain fatty acyl TG, DG, and MG) and O-PC. Reasons for the differences in the lipid concentrations, mainly for glycerolipids comparing heparin 3 and EDTA for individual volunteers, may be the blood collection tube or the blood drawing procedure as the collection was performed at the same time. The calculated p-values for all samples and for each gender separately show no statistical significant differences between EDTA and heparin plasma, except for MG 18:1 and some PC (Table S-9 and Table S-10), indicating that the lipidome obtained for EDTA and heparin plasma are overall similar.

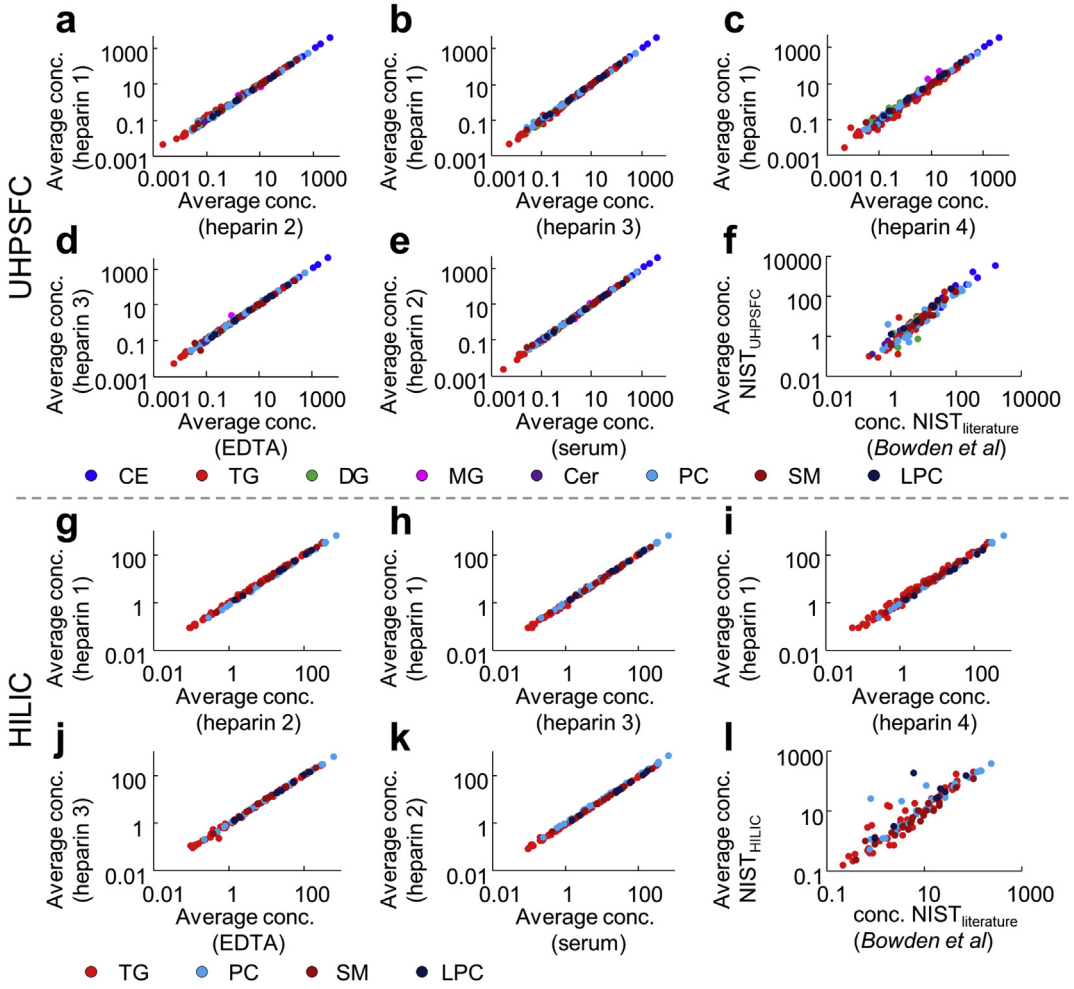


Fig. 2. Correlation plots for the lipid concentrations determined in human blood; the mean concentration from all samples per sample type was used-using heparin lithium blood collections tubes at different time points for UHPSFC/MS a) and b) and for HILIC-UHPLC/MS g) and h), where heparin 1 corresponds to the 1st collection, heparin 2 to the 2nd collection after 6 months and heparin 3 to the 3rd collection after 12 months. The influence of sample collection sites and subjects is illustrated in c) for UHPSFC/MS and i) for HILIC-UHPLC/MS, as heparin 4 was collected at a different place from different volunteers. The influence of the collection tube type is illustrated in d) and e) for UHPSFC/MS and j) and k) for HILIC-UHPLC/MS, where heparin 3 and EDTA as well as heparin 2 and serum were collected at the same time. The difference of lipid concentrations obtained with f) UHPSFC/MS and l) HILIC-UHPLC/MS in comparison to literature [25] is illustrated.

3.5. Comparison of serum and heparin plasma

The systematic comparison of lipid concentrations in plasma and serum of the same volunteers was carried out. At the 2nd blood collection, serum samples were collected for the 99 volunteers in parallel to heparin plasma. Mean concentrations of individual lipid species for all volunteers were calculated for serum and plasma (Table S-9 and Table S-10). Correlation plots show that lipid concentrations are comparable for both sample types, independent of the method employed (Fig. 2e for UHPSFC/MS and Fig. 2k for HILIC-UHPLC/MS). However, slightly higher concentrations (*ca.* 20%) were observed for serum samples compared to the plasma samples. The RSD for serum and heparin plasma for the same volunteer were calculated for UHPSFC/MS and HILIC-UHPLC/MS (Table S-5 and Table S-8). The RSD was <20% for 80% of all lipids in all volunteers using UHPSFC/MS or 73% for HILIC-UHPLC/MS. In general, a higher deviation is observed for MG, DG, the short fatty acyl TG, and O-PC. Increased RSD for all lipid species are observed for few donors (but excluding IS), which suggests some issues during the blood drawing or serum/plasma isolation. The p-value calculated for all samples and individual genders revealed only statistical significant differences in the lipid concentrations obtained in plasma and serum for LPC 16:0 using HILIC-UHPLC/MS, and for MG 18:1, PC O-40:1, LPC 16:0 and LPC 18:0 for UHPSFC/MS. The observation that lipid concentrations are higher for serum in comparison to plasma is in consistent with fomer studies reported by Yu et al. [7] and Liu et al. [3], who investigated metabolite profile differences of serum and EDTA plasma. Aoki et al. reported elevated levels of some LPC in serum due to released phospholipases by activated platelets during coagulation [23]. Generally, results indicate that the time points have a higher impact on the lipid concentration profiles than the used collection tubes, such as EDTA plasma, heparin plasma, or serum. However, independent of the time period and sample collection tube, as general trend, it is observed that the glycerolipids MG, DG and TG as well as PC-O are more prone to concentration changes than the other lipid classes, which might be considered in future blood biomarker studies.

3.6. Influence of the sample collection site

Third parties, such as clinics or biobanks, often provide samples for clinical research studies at universities, where the sample collection site may differ due to the merging from several departments. Therefore, the influence of the sample collection site on

lipid concentrations was investigated. The lipid concentrations obtained for 99 samples from the 1st blood collection (heparin 1) were compared to 81 heparin plasma samples (heparin 4) obtained from the University Hospital Olomouc from different cohort of healthy volunteers. The mean lipid concentration of each sample collection site was calculated and compared. Correlation plots indicate that especially the concentrations of triacylglycerols vary for samples from different origin (Fig. 2c and i). For 30% of lipids, the RSD of mean lipid concentrations for both collection sites is >10%, mainly for the triacylglycerols, diacylglycerols and monoacylglycerols (25%) using UHPSFC/MS. A similar trend is observed for HILIC-UHPLC/MS, where 38% of the lipids show the RSD >10%, whereby 30% belong to triacylglycerols, indicating that lipid concentrations belonging to other lipid classes CE, SM, PC, LPC, and Cer are highly comparable between different sample collection sites independent of the method employed. As the TG IS, not used for quantitation, has an RSD <10% for both methods (Fig. S-2), the concentration differences of the glycerolipids are not caused by a measurement bias, but potentially by exogenous factors like nutrition, fasting state and activity level of the volunteers. However, as the concentration differences for glycerolipids were observed for the collection site, the time period and the different blood collection tubes, it is assumed that pre-analytical factors like the blood drawing procedure may also affect glycerolipid concentrations to a certain extent. Further investigations on the influence of pre-analytical steps on the concentrations of glycerolipid species are necessary for confirmation, but out of scope for this study. Phospholipids and sphingolipids are, among others, signaling molecules and may be important representatives for various pathological states. It is of importance to know that phospholipid and sphingolipid concentrations are independent of the sample collection site and therefore applicable for clinical studies investigating pathological disorders.

The mean concentrations in the heparin plasma samples from the 1st collection were also compared to mean lipid concentrations obtained for the NIST plasma. The NIST plasma is a pooled sample of 100 humans collected after overnight fasting. The NIST plasma was gender and age matched to the racial distribution representing the distribution of the US population (77% Caucasian, 12% African American, 2% American Indian, 4% Asian, and 5% others). The heparin 1 was also collected in the morning after overnight fasting. Correlation plots of mean lipid concentrations measured for heparin 1 and NIST plasma show a higher deviation for all lipid classes independent of the employed method (Fig. S-3). For HILIC-UHPLC/MS, lipid concentrations for heparin 1 are overall higher than for the NIST plasma. 79% of all lipids have the RSD >10% calculated for the mean NIST plasma and heparin 1 lipid concentrations for both methods. Results indicate that the cohorts obtained within the Czech Republic are homogenous, but show pronounced differences compared to NIST plasma samples collected in the USA (Fig. 5c, Table S-9, and Table S-10). Lipidomic concentration changes depending on the ethnicities were reported before by Saw et al. [24], who identified 107 lipid species belonging to 15 lipid subclasses in human plasma differentiated between 3 populations (Chinese, Malay and Indian). The observation that the geographic origin of samples, probably associated with cultural differences and nutritional habits, can significantly affect the blood lipidome may be of relevance for future studies.

3.7. Comparison of the quantitation of NIST plasma using HILIC-UHPLC/MS or UHPSFC/MS with literature data

The mean concentration of lipids in NIST plasma obtained with UHPSFC/MS and HILIC-UHPLC/MS was compared to the consensus values [25]. The correlation plots indicate a high deviation of lipid concentrations for NIST plasma and values reported in the literature (Fig. 2f and l). 19% (UHPSFC/MS) and 26% (HILIC-UHPLC/MS) of the lipids have the RSD <10%, 29% and 39% of lipids have the RSD between 10 and 25% and 52% and 35% have the RSD >25% for NIST plasma measured with UHPSFC/MS and HILIC-UHPLC/MS, respectively, in comparison to literature consensus values. Lipid concentrations for the NIST plasma measured with HILIC-UHPLC/MS correspond better with literature values than UHPSFC/MS results. Consensus values were obtained by the calculation of the median of laboratory means from more than 30 laboratories. However, different sample preparation protocols, quantitation approaches (including GC/MS, UHPLC/MS, and DI-MS in targeted or untargeted manner) and IS were used. Differences in lipid concentrations between literature consensus values and the present results may have multiple reasons including the heterogeneity in sample preparation protocols, quantitation approaches, applied methodologies, *etc.,* and this situation underscores the need of harmonization of lipidomic workflows suggested by the International Lipidomic Society [26].

3.8. Comparison of all sample types

RSD of mean lipid concentrations for all sample types, like the different time points (heparin 1, heparin 2, and heparin 3), the different sample collection sites (heparin 4), EDTA plasma, and serum were calculated for HILIC-UHPLC/MS and UHPSFC/MS. RSD <20% was observed for most of the lipid species independent of the employed method (Fig. 3e, Fig. 3f, Table S-9, and Table S-10). Short fatty acyl chain TG and MG have higher RSD calculated for all sample types. The RSD together with boxplots show that the influence of time and the blood collection tube used on overall concentrations for majority of lipids is minor (Fig. S-1), as illustrated on the example of SM 41:1 (Fig. 4a, Fig. S-4, and Fig. S-5). The PCA-X statistical model for the mean concentration of lipid species measured with both methods per sample type also reflects that sample types are comparable, due to the clustering (Fig. 5c). Furthermore, the PCA demonstrates the lipidomic concentration differences of the NIST plasma and literature values in comparison to the other sample types, as the points are apart from the others (see Chapter 3.6 and 3.7). The PCA also shows clear clustering of the sample types depending on the method employed, suggesting quantitation performance differences are dependent on the method employed.

The RSD for all samples within the same sample type for each lipid species was calculated, describing the biological variability. Afterwards the mean of those RSDs were calculated for each lipid species. The mean RSD <10% was only observed for exogenous IS, all others were >20% (Fig. 3g and h). This clearly indicates that the biological variance of lipids is relatively high, in contrast to the measurement variability. The extent of the biological variance is much higher than the variance of different sample types. Reasons for the high biological variance can be versatile, like fasting state, nutrition, activity level, health state, hormone cycle, circadian rhythm. Therefore, it seems essential to use a sufficient number of subjects for clinical research studies in order to determine specific lipidomic profiles depending on a pathological state.

3.9. Comparison of methods

HILIC-UHPLC/MS and UHPSFC/MS were used for the lipidomic analysis of different sample types. The same sample extracts were measured with both methods, and the same sequence template was used. However, samples were freshly diluted for each method. The principal difference between these methods is that non-polar lipid classes and ceramides elute in the void volume of the

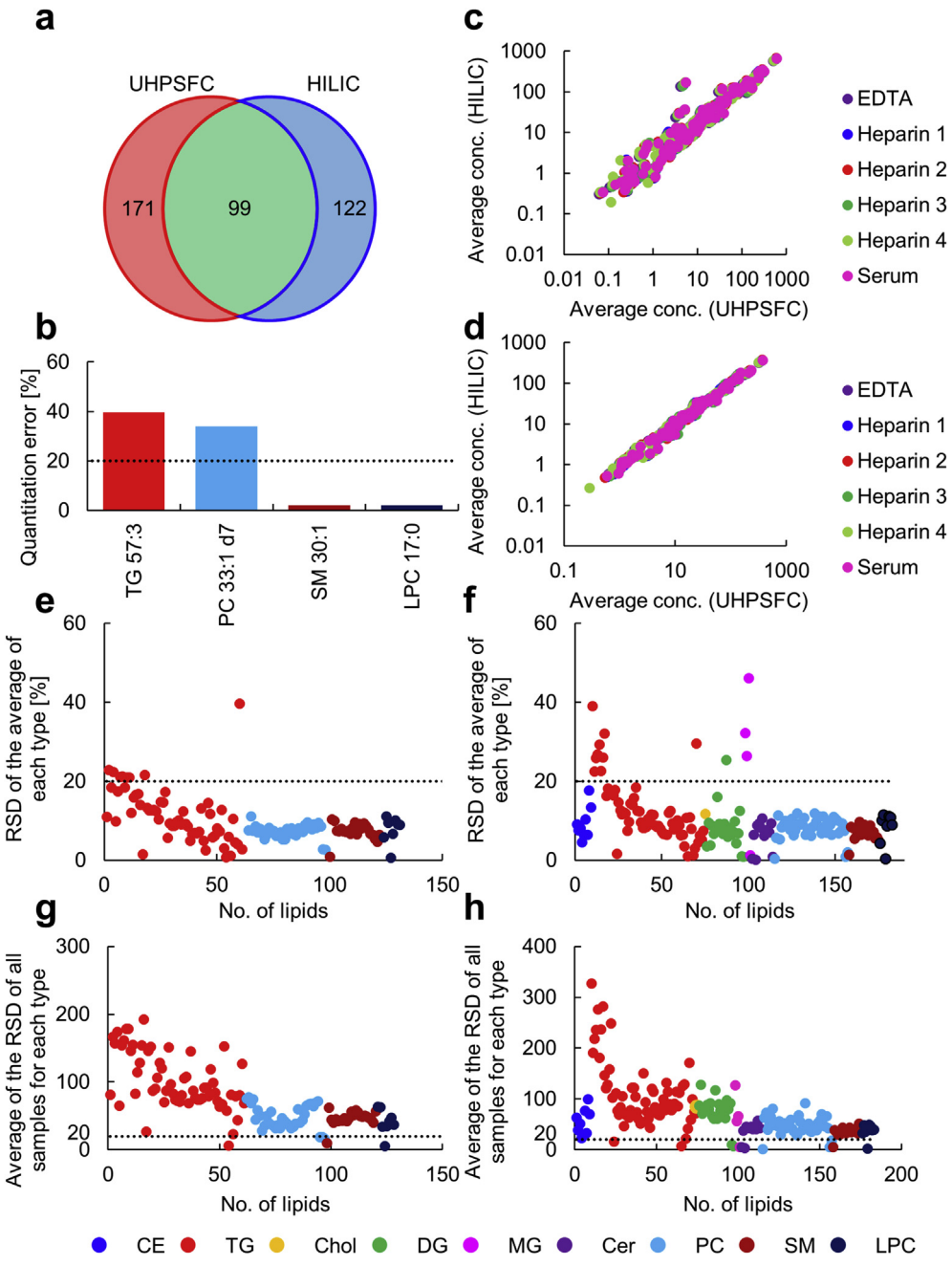


Fig. 3. a) Venn diagramm of quantified lipids with UHPSFC/MS and HILIC-UHPLC/MS; b) quantitation error of the different lipid classes in HILIC-UHPLC/MS c) correlation plot of mean lipid concentrations determined for the different blood collection tubes and time points before normalization using UHPSFC/MS and HILIC-UHPLC/MS d) correlation plot of mean lipid concentrations determined for the different blood collection tubes and time points after normalization using UHPSC/MS and HILIC-UHPLC/MS e) RSD calculated from the mean concentration of each type of blood collection tube, time point and collection place for HILIC-UHPLC/MS f) RSD calculated from the mean concentration of each type of blood collection tube, time point and collection place for UHPSFC/MS. Mean of the RSD obtained from the different samples per sample type for g) HILIC-UHPLC/MS and h) UHPSFC/MS.

system in case of HILIC-UHPLC/MS. Therefore, the quantitation of CE, DG, MG, and Cer is not recommended for HILIC-UHPLC/MS, because quantitation errors may occur due to in-source fragmentation and interferences. 122 lipid species belonging to the classes TG, PC, SM, and LPC were quantified with HILIC-UHPLC/MS (Figs. 3a), and 171 lipid species were quantified with UHPSFC/MS excluding the multiple IS, whereby 99 lipid species were quantified with both methods (Fig. 3a). The quantitation error is higher for TG, PC, and LPC and lower for SM with HILIC-UHPLC/MS (Fig. 3b) than with UHPSFC/MS (Fig. 1h). The correlation plots for lipid concentrations comparing UHPSFC/MS and HILIC-UHPLC/MS for all sample types (Fig. 3c) show differences in quantitation results. The RSD is <10% for about 19%, between 10 and 25% for about 34%, 25–40% for about 23%, and >40% for about 24% of the lipid species considering all sample types (heparin 1, heparin 2, heparin 3, heparin 4, EDTA plasma, serum, and NIST plasma). Generally, the lipid concentrations are higher for HILIC-UHPLC/MS in comparison to UHPSFC/MS. The splitting of the flow and therefore sample amount reaching the mass spectrometer, the overall higher flow rates and the addition of a make up solvent, paired with gradient elution may affect electrospray ionization using UHPSFC/MS resulting in altered ionization yield. Differences in the matrix effect, ionization effects, the

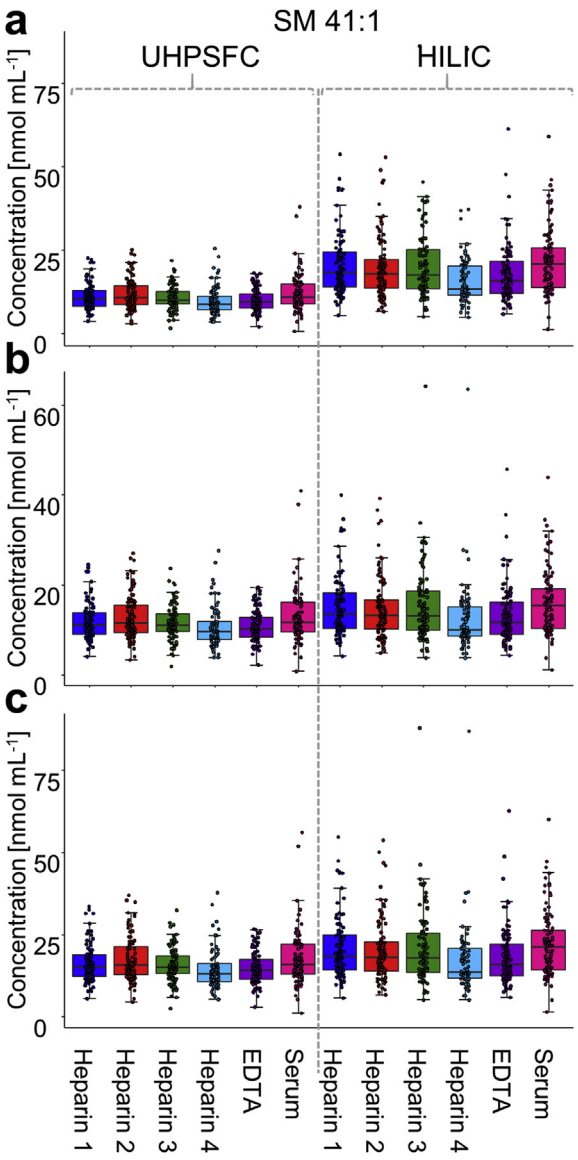


Fig. 4. Boxplot of SM 41:1 a) comparison of the SM 41:1 concentrations determined for the various time points and different blood collection tubes using UHPSFC/MS and HILIC-UHPLC/MS b) comparison of the SM 41:1 concentrations obtained for various time points and blood collection tubes after normalization with the NIST plasma and conversion to absolute quantities using the lipid concentration values from literature data [25] c) comparison of the SM 41:1 concentrations obtained for various time points and blood collection tubes after normalization with the NIST plasma and conversion to absolute quantities using the lipid concentration values of mean data published previously for UHPSFC/MS [20].

stability of instruments, and contamination issues are additional potential reasons. Differences in lipid concentrations for both methods have to be caused by the measurement itself, because the same sample extracts were measured. The data normalization may diminish lipid concentration differences caused by measurements.

3.10. Normalization

The normalization procedure was performed in line with recommendations from the previous report [27] for individual sample types separately. Mean lipid concentrations determined in this study were divided by corresponding lipid concentrations obtained by us for the NIST plasma, and then multiplied with reported reference values [25,27]. Boxplots (Fig. 4) illustrate that the normalization significantly helps to diminish lipid concentration differences for two methods used in this work. As multiplier either concentration values reported by Bowden et al. [25] (Figs. 4b and 3d) or the mean of plasma, serum and NIST plasma obtained during method validation [20] (Fig. 4c) for UHPSFC/MS were used. It can be seen that both multiplier are applicable for quantitation, but it is hard to conclude which quantitation results are closer to trueness. The current efforts of the lipidomic community to establish consistent and reliable consensus values, obtained by multiple laboratories under given conditions like the use of a unified sample preparation protocol, the same quantitation approach and analysis technique, may significantly improve quantitation results in the future [26], as those values can be used as multipliers to obtain absolute quantities after normalization [27]. In the following, absolute values after normalization were obtained by using the reference values from Bowden et al. [25].

The mean of lipid species concentrations obtained with UHPSFC/MS and HILIC-UHPLC/MS was calculated for the mean of each sample type. The deviation from average was calculated by subtracting the mean concentration of lipid obtained by the particular method from the mean for both methods and dividing by the mean for both methods. This was performed for all sample types, for both methods, before and after the normalization. Plotting the deviation from the mean against the concentration shows the quantitation bias obtained for both methods. The quantitation bias for EDTA plasma is illustrated before (Fig. 5a) and after (Fig. 5b) the normalization. The normalization reduces the method-dependent quantitation bias. The normalization completely removed the method-dependent bias and provides highly coherent quantitative data for two different quantitation methods, as illustrated in the PCA-X (Fig. 5d). This supports the conclusion of previously published work [27] that normalization helps to reduce method dependent bias and allows the comparison of lipidomic results obtained for intra- and inter-laboratory studies. Consequently, it is recommended to measure regularly the NIST plasma during lipidomics studies, which allows the normalization and comparison to other methods.

4. Conclusions

We summarize here recommendations for the quality assurance in lipidomic studies based on the lipid class separation (UHPSFC/MS and HILIC-UHPLC/MS), including the use of the independent randomization for the sample preparation and measurements, the monitoring of the instrumental performance and sample preparation during measurements, the use of appropriate QC samples and more than one IS per lipid class. This practise is advisable for any lipidomic quantitation workflow. The accuracy of quantitation, the number of identified lipid species, and the instrumental stability in the present study yield better results for UHPSFC/MS in comparison to HILIC-UHPLC/MS. Samples collected for the same subjects at the same place but using different collection tubes (heparin plasma, EDTA plasma, and serum) provide similar lipidomic profiles, but lipid concentrations in serum are about 20% higher in comparison to plasma. It is very unlikely that the utilization of different collection tubes would be planned in prospective studies, but in retrospective studies samples may be obtained from different places using different blood collection tubes. From this perspective, it is important to demonstrate that differences can be rather small if the right practice is followed at all sites. The lipidomic profiling of heparin plasma samples obtained from three blood collections during one year, illustrating the biological variability over time, revealed altered concentrations of the non-polar lipid species. For the future progress in clinical lipidomics, the harmonization of

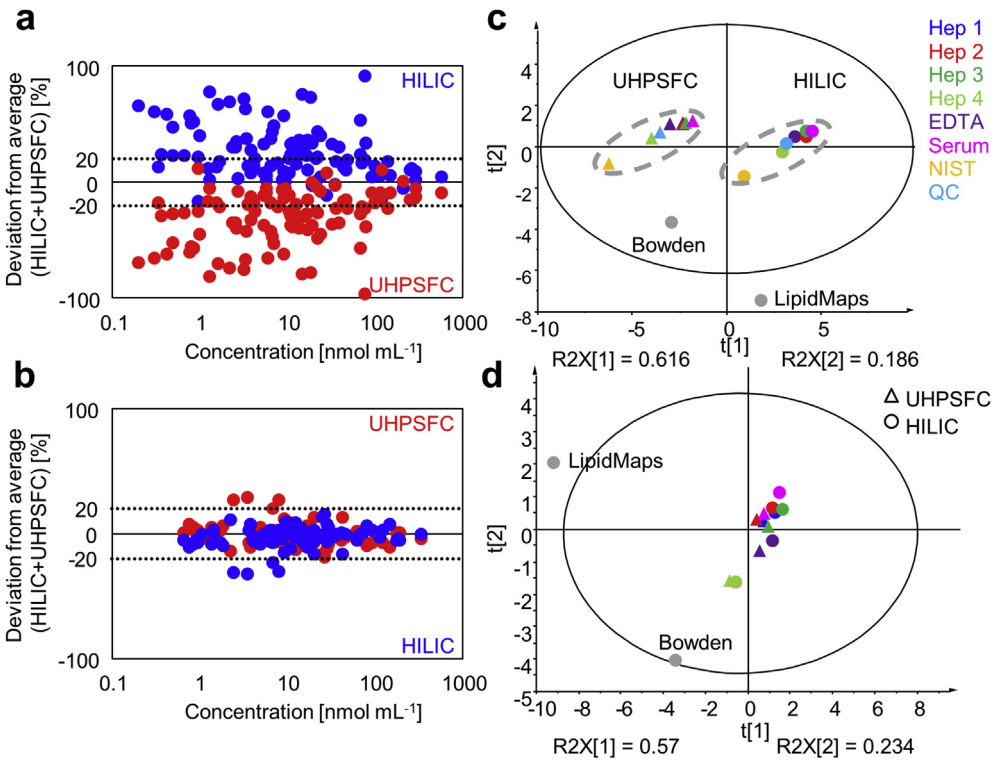


Fig. 5. a) Deviation from the average of HILIC-UHPLC/MS and UHPSFC/MS for the mean lipid concentrations determined for EDTA collection tubes using UHPSFC/MS or HILIC-UHPLC/MS. b) Deviation from the normalized mean of HILIC-UHPLC/MS and UHPSFC/MS for the mean lipid concentrations determined for EDTA collection tubes using UHPSFC/MS or HILIC-UHPLC/MS after normalization. The normalization was perfomed by dividing the mean lipid species concentration of all samples for one sample type and the mean of the NIST plasma, followed by the conversion to absolute quantities by multiplying with the lipid concentrations from literature [22] c) PCA of the mean lipid concentrations obtained for the different sample types with HILIC-UHPLC/MS and UHPSFC/MS. d) PCA of the mean concentrations obtained for the different sample types with HILIC-UHPLC/MS and UHPSFC/MS after normalization.

reference concentration ranges for the healthy population and the NIST SRM1950 reference plasma using molar concentrations is urgently needed, which should help the translation of lipidomic methods into the real-world clinical practise together with the best practise for the data integrity recommended in this work.

Funding information

This work was supported by the junior grant project 20-23290Y funded by the Czech Republic Science Foundation.

Compliance with ethical standards

All volunteers signed informed consent, and the ethical committee approved the blood collection.

CRediT authorship contribution statement

Denise Wolrab: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Funding acquisition. **Michaela Chocholoušková:** Investigation, Writing - review & editing. **Robert Jirásko:** Visualization, Writing - review & editing. **Ondřej Peterka:** Investigation, Writing - review & editing. **Vladimíra Mužáková:** blood collection, Writing - review & editing. **Hana Študentová:** blood collection. **Bohuslav Melichar:** Resources, Writing - review & editing. **Michal Holčapek:** Resources, Project administration, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors thank all volunteers for their willingness to donate blood, and Jana Kňavová and Iva Fousová (University of Pardubice) for the help with the sample collection and processing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.08.061.

References

[1] A.-M. Simundic, et al., Joint EFLM-COLABIOCLI Recommendation for venous blood sampling 56 (12) (2018) 2015.
[2] G. Lima-Oliveira, D. Monneret, F. Guerber, G.C. Guidi, Sample management for clinical biochemistry assays: are serum and plasma interchangeable specimens? Crit. Rev. Clin. Lab Sci. 55 (7) (2018) 480–500.
[3] X. Liu, M. Hoene, X. Wang, P. Yin, H.-U. Häring, G. Xu, R. Lehmann, Serum or plasma, what is the difference? Investigations to facilitate the sample material selection decision making process for metabolomics studies and beyond, Anal. Chim. Acta 1037 (2018) 293–300.
[4] M.A. López-Bascón, F. Priego-Capote, A. Peralbo-Molina, M. Calderón-Santiago, M.D. Luque de Castro, Influence of the collection tube on metabolomic changes in serum and plasma, Talanta 150 (2016) 681–689.
[5] E. Jobard, O. Trédan, D. Postoly, F. André, A.-L. Martin, B. Elena-Herrmann, S. Boyault, A systematic evaluation of blood serum and plasma pre-analytics for metabolomics cohort studies, Int. J. Mol. Sci. 17 (12) (2016) 2035.

[6] M. Suarez-Diez, et al., Plasma and serum metabolite association networks: Comparability within and between studies using NMR and MS profiling, J. Proteome Res. 16 (7) (2017) 2547–2559.
[7] Z. Yu, et al., Differences between human plasma and serum metabolite profiles, PloS One 6 (7) (2011), e21230.
[8] J. Lan, et al., Systematic evaluation of the use of human plasma and serum for mass-spectrometry-based shotgun proteomics, J. Proteome Res. 17 (4) (2018) 1426–1435.
[9] J.R. Denery, A.A.K. Nunes, T.J. Dickerson, Characterization of differences between blood sample matrices in untargeted metabolomics, Anal. Chem. 83 (3) (2011) 1040–1047.
[10] M.K. Tuck, et al., Standard operating procedures for serum and plasma collection: Early detection research network consensus statement standard operating procedure integration working group, J. Proteome Res. 8 (1) (2009) 113–117.
[11] D.C. Wedge, et al., Is serum or plasma more appropriate for intersubject comparisons in metabolomic studies? An assessment in patients with small-cell lung cancer, Anal. Chem. 83 (17) (2011) 6689–6697.
[12] O. Teahan, S. Gamble, E. Holmes, J. Waxman, J.K. Nicholson, C. Bevan, H.C. Keun, Impact of analytical bias in metabonomic studies of human blood serum and plasma, Anal. Chem. 78 (13) (2006) 4307–4318.
[13] B.R. Leyland-Jones, et al., Recommendations for collection and handling of specimens from group breast cancer clinical trials, J. Clin. Oncol. 26 (34) (2008) 5638–5644.
[14] P. Yin, R. Lehmann, G. Xu, Effects of pre-analytical processes on blood samples used in metabolomics studies, Anal. Bioanal. Chem. 407 (17) (2015) 4879–4892.
[15] M.R. Wenk, The emerging field of lipidomics, Nat. Rev. Drug Discov. 4 (7) (2005) 594–610.
[16] D. Wolrab, R. Jirásko, M. Chocholoušková, O. Peterka, M. Holčapek, Oncolipidomics: mass spectrometric quantitation of lipids in cancer research, Trac. Trends Anal. Chem. 120 (2019), 115480.
[17] M. Holčapek, G. Liebisch, K. Ekroos, Lipidomic analysis, Anal. Chem. 90 (7) (2018) 4249–4257.
[18] R. Hájek, R. Jirásko, M. Lísa, E. Cífková, M. Holčapek, Hydrophilic interaction liquid chromatography–mass spectrometry characterization of gangliosides in biological samples, Anal. Chem. 89 (22) (2017) 12425–12432.
[19] E. Cífková, M. Lísa, R. Hrstka, D. Vrána, J. Gatěk, B. Melichar, M. Holčapek, Correlation of lipidomic composition of cell lines and tissues of breast cancer patients using hydrophilic interaction liquid chromatography/electrospray ionization mass spectrometry and multivariate data analysis, Rapid Commun. Mass Spectrom. 31 (3) (2017) 253–263.
[20] D. Wolrab, M. Chocholoušková, R. Jirásko, O. Peterka, M. Holčapek, Validation of lipidomic analysis of human plasma and serum by supercritical fluid chromatography–mass spectrometry and hydrophilic interaction liquid chromatography–mass spectrometry, Anal. Bioanal. Chem. 412 (10) (2020) 2375–2388.
[21] M. Lísa, M. Holčapek, High-throughput and comprehensive lipidomic analysis using ultrahigh-performance supercritical fluid chromatography–mass spectrometry, Anal. Chem. 87 (14) (2015) 7187–7195.
[22] Lipidomics standard initiative. https://lipidomics-standards-initiative.org/guidelines/lipid-species-quantification. (Accessed 14 July 2020).
[23] J. Aoki, et al., Serum lysophosphatidic acid is produced through diverse phospholipase pathways, J. Biol. Chem. 277 (50) (2002) 48737–48744.
[24] W.-Y. Saw, et al., Establishing multiple omics baselines for three Southeast Asian populations in the Singapore integrative omics study, Nat. Commun. 8 (1) (2017) 653.
[25] J.A. Bowden, et al., Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950–Metabolites in Frozen Human Plasma, J. Lipid Res. 58 (12) (2017) 2275–2288.
[26] G. Liebisch, et al., Lipidomics needs more standardization, Nature Metabolism 1 (8) (2019) 745–747.
[27] A. Triebl, et al., Shared reference materials harmonize lipidomics across MS-based detection platforms and laboratories, J. Lipid Res. 61 (1) (2020) 105–115.