<https://helda.helsinki.fi>

Advances in lipidomics

Avela, Henri F.

2020-11

Avela , H F & Siren , H 2020 , ' Advances in lipidomics ' , Clinica Chimica Acta , vol. 510 , pp. 123-141 . <https://doi.org/10.1016/j.cca.2020.06.049>

<http://hdl.handle.net/10138/332472>

<https://doi.org/10.1016/j.cca.2020.06.049>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry – A review

Henri F. Avela and Heli Sirén

Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 University of Helsinki
(FI-00560 Helsinki), Finland

^{*)} Correspondence: Henri F. Avela (MSc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, Finland, henri.avela@hotmail.com; Heli Sirén (Assoc. Professor, Doc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, Finland, heli.m.siren@helsinki.fi

Abstract

Lipids are organic biomolecules, which contribute to energy storing, cellular and subcellular membrane assembly, functionality, signalling, gene expression regulation and surfactant formation. Lipidomics comprises of identification and quantitation of organic lipids, their derivatives and variants.

The present review article combines the literature on glycerol-, glycerophospho- and sphingolipids in lipidomics from the years 2017-2019. In addition, a few papers which have promoted advancement in lipidomics are discussed. The primary focus is lipid profiling of biological lipidomic systems with ultrahigh performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS, tandem MS) detection, data handling, and calculations with analytical tools and current statistical methods. Research on *in vivo* samples includes matrix categories, such as primary blood derivatives (plasma, serum, erythrocytes, and blood platelets), faecal matter, urine, and liver tissues.

Keywords: Lipids, liquid chromatography, tandem mass spectrometry; clinical profiling, urine, blood.

Highlights:

Compilation of the recent literature on clinical samples; presentation of the accurate methodology.

Evaluation of sample preparation techniques in lipid analyses.

Information on separation and identification of neutral, hydrophobic and hydrophilic lipids.

Compilation of current statistical methods used in mass spectrometry of glycerol-, glycerophospho- and sphingolipids.

Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry – A review

Henri F. Avela and Heli Sirén

Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 University of Helsinki (FI-00560 Helsinki), Finland

^{*)} Correspondence: Henri F. Avela (MSc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, henri.avela@hotmail.com Finland; Heli Sirén (Assoc. Professor, Doc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, Finland, heli.m.siren@helsinki.fi

Abstract

Lipids are organic biomolecules, which contribute to energy storing, cellular and subcellular membrane assembly, functionality, signalling, gene expression regulation and surfactant formation. Lipidomics comprises of identification and quantitation of organic lipids, their derivatives and variants.

The present review article combines the literature on glycerol-, glycerophospho- and sphingolipids in lipidomics from the years 2017-2019. In addition, a few papers which have promoted advancement in lipidomics are discussed. The primary focus is lipid profiling of biological lipidomic systems with ultrahigh performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS, tandem MS) detection, data handling, and calculations with analytical tools and current statistical methods. Research on *in vivo* samples includes matrix categories, such as primary blood derivatives (plasma, serum, erythrocytes, and blood platelets), faecal matter, urine, and liver tissues.

25 **Keywords:** Lipids, liquid chromatography, tandem mass spectrometry; clinical profiling, urine,
26 blood.

27 **Highlights:** Compilation of the recent literature on clinical samples; presentation of the accurate
28 methodology; sample preparation; information on separation and identification of neutral,
29 hydrophobic and hydrophilic lipids; compilation of current statistical methods used in mass
30 spectrometry of glycerol-, glycerophospho- and sphingolipids.

31 **Contents**

32 1 Lipidomics

33 1.1 Classification of lipids

34 1.2 Lipid types

35 1.3 Lipid synthesis in organisms

36 1.4 Cellular functions of lipids

37 2 Interpretation of lipidomic profiles

38 2.1 Profiling of homeostatic systems

39 2.2 Profiling of metabolic alterations

40 2.3 Profiling of diseases

41 3 Sampling and storage for lipid analyses

42 4 Sample preparation for lipid analyses

43 5 Liquid chromatography in lipidomics

44 5.1 High-performance liquid chromatography in lipidomics

45 5.2 UHPLC systems in lipid analyses

46 6 Mass spectrometry in lipidomics

47 6.1 Mass spectrometric analyses of lipids

48 7 Method optimization

49 7.1 Lipid classes studied with liquid chromatography Voisi siirtää 5 ja 6 jälkeen?

50 8 Lipids important in clinical and medical samples

51 8.1 Lipids and diseases

52 9 Metabolite identification with MS and data analysis

53 10 Untargeted vs targeted approaches

54 11 Current statistical methods used in mass spectrometry

55 12 Discussion

56 13 Conclusions

57 14 References

58

59 **Abbreviations**

60 ACP acyl carrier protein; Cer ceramides; Cer-PC ceramide – phosphatidylcholine; Cer-PE ceramide
 61 – ethanolamine; CEs cholesteric esters; DG diglycerols; DB double bond; ESI electrospray
 62 ionization; FA fatty acid; GluCer glucosylceramide; GPs glycerophospholipids; GLs glycerolipids;
 63 HDL/LDL/VLDL high/low/very low density lipoproteins; HILIC hydrophilic interaction
 64 chromatography; LC liquid chromatography; LacCer lactosyl ceramide; LPC
 65 lysophosphatidylcholines; LPE lysophosphatidylethanolamine; MS mass spectrometry; MALDI
 66 matrix-assisted laser desorption ionization; MG mono glycerols; MGDG
 67 monogalactosyldiacylglycerol; MUFA monounsaturated fatty acids; NADPH nicotinamide adenine
 68 dinucleotide phosphate; nanoLC nano-liquid chromatography; NEFA/FFA non-esterified fatty
 69 acids/free fatty acids; NP normal phase chromatography; PA phosphatidic acid; PG
 70 phosphatidylglycerol; PI phosphatidylinositol; PKs Polyketides; PUFA polyunsaturated fatty acids;
 71 RP reversed phase chromatography; PRLs prenol lipids; S sphingosine; SA sphinganine; SA1P
 72 sphinganine-1-phosphate; SFA saturated fatty acids; SLs saccharolipids; SM sphingomyelin; SPs
 73 sphingolipids; SQDG sulfoquinovosyl diacylglycerols; ST cholesterol-based sterols; S1P
 74 sphingosine-1-phosphate; PC phosphatidylcholines; PE phosphatidylethanolamine; TG triglycerols;

75 TGDG trigalactosyldiacylglycerol; UHPLC-MS ultrahigh performance liquid chromatography-mass
76 spectrometry.

77 **1 Lipidomics**

78 Lipidomics is a subgroup within the field of metabolomics with which lipid metabolites can give
79 valuable information about organisms and their biological functions. Lipidomics may be divided
80 into membrane-lipidomics and mediator-lipidomics, [1] which include either comprehensive or
81 quantitative description of membrane lipid constituents or structural characterization and
82 quantification of low abundant bioactive lipid species, respectively. As a sector of metabolomics
83 devoted to the qualitative and quantitative analyses of the lipidome, lipidomics has met an utmost
84 expansion to various biochemical areas. [2] Lipids may be involved in explaining the physiology of
85 multiple interlinked organs, a single organ, biological tissues, individual cells, and biofluids.

86 In broad terms, lipidomic research covers all aspects of individual lipid species including their
87 structure, function, connection with other cell constituents such as protein, lipid, and metabolite in
88 both health and disease conditions. Details of cell biology obtained from different pathogens
89 (viruses, bacteria, and parasites) provide a great amount of data on molecular structure of host
90 pathogen relations and, consequently, on infection processes [3]. Novelty of lipid analytics is related
91 to reliable, accurate, and sensitive analytical methods for observing new lipid information and
92 correlation with diseases. Abnormalities in lipid metabolism may be an indicator for the stage of a
93 lipidome-related disease, a correlation factor which may be observed when comparing a patient
94 with a healthy individual [4] [5].

95 The current review describes identification and determination of lipids in biological, clinical and
96 medical samples with ultrahigh performance liquid chromatography – mass spectrometry (UHPLC-
97 MS) in 2017-2019. The publications discuss about determination of biological samples based on (1)
98 chromatography/separation, (2) identification with various types of mass spectrometry, (3) matrices,
99 (4) lipid groups, and (5) mathematical data handling.

100 **1.1 Classification of lipids**

101 According to the definition of lipids made by Fahy et al. [6] lipids are “hydrophobic or amphipathic
102 small molecules that may originate entirely or in part by carbanion-based condensations of
103 thioesters and/or by carbocation-based condensations of isoprene units”. [6] Recently, the newest
104 recorded updates of the definition was published by the lipidomics gateway LIPID MAPS®
105 consortium (https://www.lipidmaps.org/data/classification/LM_classification, March 20th 2017).
106 Thus, lipids are characterized according their backbone structure. Lipids include
107 glycerophospholipids (GPs), glycerolipids (GLs), sphingolipids (SPs), polyketides (PKs),
108 cholesterol-based sterols (ST) such as cholesteric esters (CEs), prenol lipids (PRLs) and
109 saccharolipids (SLs). In addition, high-/low-/very low-density lipoproteins (HDL/LDL/VLDL) are
110 frequently studied alongside conventional lipids, though lipoproteins are excluded from this study.

111 **1.2 Lipid types**

112 Polar fatty acids (FA) contain fundamental elements of more complex lipids (e.g. for
113 triacylglycerols). They are heteroatom-substituted and esterified conjugates, e.g. acyl carnitines
114 [AcCA] [7]-[10] or bis(monomyristoylglycero)phosphate [BMP] [11], which may include branched
115 or cyclic functionalities [4]. In comparison to the lipid headgroups and hydrocarbon backbones, FA
116 chain function and diversity is less understood. Antonny et al. [12] report several FA saturation
117 gradients: saturated fatty acid glycerophospholipids (SFA GPs) increase the further they are
118 processed in the mammalian cell, namely from the endoplasmatic reticulum (ER) to Golgi apparatus
119 to plasma membrane. [12] Conversely, neural cells have rare sightings of poly-unsaturated fatty
120 acid phosphatidylcholines (PUFA PCs) around the soma, increasing towards the C20:4- and C22:6-
121 rich PCs axon end. Higher saturation is often present in uptake-based long FA chains like C22:6-n3,
122 synthesized from C18:3-n3 plant oil (*Figure 1*). [12]
123 Lipids get their FA chains either *de novo* via the Kennedy pathway synthesis [13] or through re-
124 acylation (i.e. FA substitution) with available components via the Lands cycle [13]. [12] This

125 substitution is enabled through acyltransferase activity whose rate of specificity affects acyl chain
 126 diversity.[12] More research on SPs with >C18 FAs is needed. For instance, counter to common
 127 belief, SM and Cer include a large abundance of C24:1 and C24:0 FAs. [15]

128 Glycerophospholipids (GPs) are derived from glycerolipids by substituting a phosphate-linked end-
 129 group to the sn3-carbon of glycerol. GPs have also passive roles as the lipid bilayer cell membrane
 130 components. The most abundant lipids in eukaryotic cells are phosphatidylcholines (PC),
 131 phosphatidylethanolamine (PE), and their derivatives. [4] Other GP sub-groups include
 132 phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerols
 133 (PG), and PG-derived cardiolipins (CL). [4]

134 GPs in FAs may include ester or ether groups, of which the latter group is defined as plasmalogens
 135 (PLs). They cover approximately one fifth of the glycerophospholipids in eukaryotic organisms. [4]
 136 PLs are divided into two categories: plasmanyls and plasmenyls. Plasmanyls are noted to have an
 137 oxygen bridge (e.g. PI[O-18:0/17:0]) or due to a phosphorus bridge in a case of a plasmenyl group
 138 with an ester bond conjugated to a double bond of the FA (e.g. PI[P-18:0, 17:0]). [4] GL and GP
 139 structures are depicted in *Figure 2* with a list of typical FAs in the mammalian lipidome.

140 Sphingolipids (SP), characteristic for eukaryotic cells, [15] have a sphingosine base as the backbone
 141 (*Figure 3*). One of these sub-classes are ceramides (Cer), which are lipidic sphingoid bases having
 142 nitrogen bonds. One of the SPS is sphingomyelin (SM), which is an interrelated [15] phospho-SP
 143 structure that combines ceramide with phosphatidylcholine (Cer-PC) or -ethanolamine (Cer-PE).

144 **1.3 Lipid synthesis in organisms**

145 Most lipids are either synthesized in the endoplasmatic reticulum, in Golgi apparatus, and in the
 146 mitochondria. [11] Glycerolipids (GL) are mono- (MG), di- (DG) or trisubstituted (TG) glycerols,
 147 which belong to common fatty acids having esterifies substituents. [18]

148 Lipids are primarily formed in FA synthesis with excess of acetyl coenzyme A in presence of
 149 NADPH [18] in a cell's cytoplasm. [19] In a model proposed by Nelson and Cox, [18] the synthesis

is a six-step process that involves most notably derivatives formed by acetyl coenzyme A and the acyl carrier protein (ACP). As for the reactants, an initial thioester carbon chain is modified to a malonyl body, which is further lengthened through a catalysed loop of acetyl group addition. The loop includes catalysed reduction steps of thioesters keto-groups (and the leftover double bonds) with NADPH and concludes with releasing of the synthesized FA conjugate base from ACP (either fully saturated or unsaturated). [20] According to Dennis, [18] the formed FAs are typically esterified further near hydroxyl groups in other biomolecules (e.g. glycerol), since the process stabilizes reactive FA. [18] The FA synthesis is biological basis for complicated lipids.

GP metabolism is considered to originate from an enzyme-assisted combination of a FA-metabolism based metabolite (acyl coenzyme A) combined with modified glycolysis products (Dihydroxyacetone phosphate and glycerol), which produces lyso-PA (LPA) further synthesized into PA. [21] As a sidenote, another pathway not going via LPA and PA synthesis is known for synthesizing plasmalogens (PL) [22] and the platelet activating factor (PAF) [23].

For SP synthesis, Cer produced in the ER is translocated and further processed in the Golgi apparatus to produce other SPs. [11] The often identified and quantified sphingosine (S), sphinganine (SA) and their phospholipid metabolites (S1P, SA1P), both of which are important in signalling and central processes alongside LPA and other lipid mediators [24], are considered to be highly potential biomarkers in disease and cancer research. [24]-[26] Pukolski-Gross et al. [26] and Haynes et al. [27] report S1P as the crossroads between reversible dephosphorylation and irreversible catabolism of SP-originated sphingosines into phosphoethanolamine and hexadecenal (in the case of SA1P, hexadecanal), catalysed by sphingosine phosphate lyase. [28] Other sources for more SP metabolism can be found from Kita et al. [24], Sahu et al. [29] and Merrill Jr. et al. [30]. More specifically, articles on Cer metabolism is available by Zheng et al. [31], t'Kindt et al. [32], Castro et al. [33] and Manni et al. [15].

1.4 Cellular functions of lipids

175 Intra- and intercellular lipid metabolism must be understood in its localized context to understand
176 their function in more complex biological systems, e.g. tissue and biofluids. Lipidomic studies often
177 discussed the matters of single cell metabolism and tissues separately, the only link between them
178 being studies on cell signalling [84]. For appropriate tissue analysis, lipid cell function must be
179 understood before drawing conclusions of in-depth lipidomic mechanisms. According to Han^[16],
180 more lipidomic pathway flux studies (i.e. reaction rate, metabolic hierarchy and interrelation
181 determinations) are necessary for better understanding lipidomic processes in living organisms^[16].
182 For example, the quality of lipids (e.g. lipid class, FA saturation and chain length) in a plasma
183 membrane promote specific lamellar phase properties such as intermolecular order, translational
184 diffusion and elasticity of a cell [11]. A change in these properties is strongly proposed to enable
185 cellular processes like cell budding and tubulation [11] as well as cell fusion and fission. [11], [12],
186 [16]

187 Van Meer et al.'s [11] extensive reviewed compiled lipidomic observations of cell organelles and
188 membranes in mammals and yeast. Cytoplasmal and extracellular leaflets of double layer
189 membranes have different lipid concentrations and permeabilities: where ER leaflets allow free
190 lipid translocation along its double layer with non-specific lipid transporters, Golgi, plasma
191 membranes and endosomes have restricted lipid mobility [11]. This leaflet asymmetry of specific
192 lipids is proposed to promote special properties for the plasma membrane in question. For
193 translocation of some "trapped" subclass species, lipids are modified enzymatically to enable leaflet
194 flipping (e.g. cytoplasmic leaflet PC is converted to DG in the Golgi apparatus) [11].

195 Van Meer et al. [11] lists out mechanisms for various means of lipid transportation: lipids can
196 diffuse either within an organelle via the double membrane-specific translocation between leaflets
197 or moved between organelles via tubule, vesicles or nonspecific/specific transport proteins (e.g. the
198 ATP-binding cartridge (ABC) exporters for GP/SP and CERT for Cer-specific transportation) [11].
199 Basically, eukaryotic membranes are most abundant in PCs (>50%) and PEs as well as PSs, PIs and

200 PAs [11]. For major component SPs, SMs are characteristic for mammalian membranes.^[16]
 201 However, this trend in GPs and SPs is not the case in yeast or other microorganisms since their
 202 metabolic pathways differ from eukaryotic [11]. For topical reasons, mammalian metabolism is
 203 primarily discussed in this review.

204 Relative to other organelles, the plasma membrane has a large abundance of SPs, [11] most of
 205 which contribute to cell signalling. These lipid classes are either synthesized in the Golgi (SPs)
 206 [11]. In addition to its barrier function, the plasma membrane is responsible for cell signalling. [11]
 207 Most of the lipids are imported The endoplasmatic reticulum (ER) is the main cell organelle for the
 208 bulk synthesis of lipids, especially GPs and cholesterol. [11] Thus, ER is interconnected with many
 209 other organelles that synthesize specific lipids [11], [16], [29], [35]: for example the Golgi
 210 apparatus. [11] Mostly known as a lipid- and protein-sorting organelle, Van Meer et al. [11] reports
 211 the Golgi to be the biggest producer of SPs [11] which are synthesized from ER-produced
 212 intermediate Cer. [11] Mitochondria are known for the unique production of cardiolipins (CL), a
 213 lipid class proposedly taking part in oxidative phosphorylation. [11], [16] Furthermore,
 214 mitochondria are also able to produce LPA for TG, PA and PG synthesis. [11]

215 Lipid droplets (LDs) are monolayered organelles responsible of TG and cholesterol supply (bound
 216 with deployable FFA) [36] for lipid regulation [36] and synthesis as well as energy storage
 217 [35],[37],[36], [11]. In addition, LDs are proposed to store lipid signalling precursors and and
 218 hydrophobic vitamins. [37] Histologically, LDs consist of a TG and steryl/retinal ester core[36] and
 219 phospholipid (PC, PE, PI, LPC, and LPE) membrane. [36] Furthermore, PC availability and lack of
 220 its key regulatory enzyme, phosphocholine cytidyltransferase- α (CTP, Kennedy pathway) [13],
 221 are considered integral regulators in LD-growth and -fusion. [35] Since some proteins (articles
 222 listed by Fujimoto and Parton) [35] are shared between ER and LDs, [11] not to mention the
 223 metabolic regulation between ER-synthesized PA and LD-stored TG usage for lipid synthesis, [37]
 224 the two cell organelles are often observed very close to each other. LDs are either positioned in

proximity, intercalated or membrane stalk-connected with the ER. [35] Similar proximity with LDs has been observed with other lipid-producing organelles [35], [36]: Welte [36] proposes nuclear LDs to affect lipid synthesis via the nucleus, suggesting that LDs regulate transcription factor, enzyme and chromatin availability [36]. Welte [36] also reports LD interaction with mitochondria and peroxisomes [16], [35], both of which are capable of β -oxidation and FA breakdown [36]. Other studied cell organelle lipidomes include endocytic pathways, namely early and late endosomes, [11] and lysosomes [38].

2 Interpretation of lipidomic profiles

2.1 Structural identification

Isobaric interference, i.e. co-isolation [39] by co-elution of species with identical or nearly the same molar mass, affects both identification and quantitation in LC-MS [40]. Thus, lipidomic structure profiling and species validation with LC-ESI-MS is mostly done with tandem mass spectrometry (MS^n) where more information on class- and species-specific fragmentation can be attained.

For instance, Ivanova et al. [41] reported the characteristic fragmentation of three major lipid classes in positive (*Figure 4*) and six major lipid classes in negative ESI mode [41]. In positive ESI mode, PC, PS and PE species experience characteristic neutral loss of headgroups as well as fragmentation into lysophosphatidic species. [41] Since negative ESI mode fragmentation seems more complex -maybe more of it is known compared to positive mode- we suggest to read further into GP fragmentation literature by Pi et al. [42] and Ivanova et al. [41] and SP fragmentation by Sullard et al. [43] and Merrill Jr et al. [153] (SP fragmentation depicted in *Figure 5*).

As a charged particle is moving against an inert gas with known velocity, its movement can be rotationally averaged. Ion mobility spectrometry (IMS) observes this by recording the drift time of an ion species in an electric field. [44] Hence the theoretical CCS value of the analytes can be measured via their drift times with no calibration standards necessarily needed. [44] Some QTOF-instruments are equipped with an IMS module, [45]-[48] enabling further identification tactics with

another molecular property dimension. However, identifying CCS values of molecules in ion mobility-mass spectrometry mode (IM-MS mode) decreases the amount of ions ending up at the TOF, which in turn decreases sensitivity in the analyzer. IM-MS may provide valuable information that is otherwise difficult to obtain, since that ion mobility is affected by head group geometry (which is impacted by phosphorylation and glycosylation). [49]

2.2 Profiling of homeostatic systems

Determining an organism's lipidome in homeostasis may both act as a fingerprint to the species in question, a distribution of traits in a population as well as a baseline or reference for possible changes of the organism's state. All articles concerning such research were listed in *Table 1*. As an example, Meulebroek et al. [50] developed a method which may cover all eight lipid classes via faecal matter, using a polarity-switching UHPLC-interfaced Orbitrap. With this, healthy controls and type 2 diabetes patients were monitored and their lipidomic profiles compared with each other. [50] Instead of interpreting as many lipid classes as possible, Manni et al. [15] focused selectively on multiple tissue's and cell culture's 12 Cer:s [51] and sphingomyelins (SMs) in positive mode. Tissue types in this targeted approach included adipose tissue (human, rat), liver (dog, human, rat, mouse), brain (rat, mouse) and serum (human, rat, mouse). [15] For more exotic lipid classes, Drotleff et al. [52] identified and quantified ST hormones from plasma, and, similarly, Gobo et al. [53] cerebral prostaglandins from brain tissue.

Mostly, profiling of human-based biological materials use *in vitro* cell culture samples and *in vivo* clinical samples. According to literature, solid and liquid biomatrices, as well as synthesized lipids [54], such as nanoscale liposomes have been studied. The *in vitro* samples included conditioned cell culture medium (CM, a suspension acquired from cells), and *in vivo* clinical samples target to e.g. to human amniotic fluid and plasma.

Ghosh and Nisala [5] showed that human tears include at least 600 identified lipids. [5] Thus, for identification and characterization of tears the samples need to be dilute and less diverse than the

aqueous bioliquids from a multi-cells and complex organisms. In their project, CMs of cell cultures (e.g. from cancer cell lines) were isolated and characterized to evaluate single lipid components in the context of a multivariate tissue. [5] Furthermore, Ghosh and Nishala showed that plasma is a complicated matrix with a large group of GPs, SLs, CEs and TGs. This is understandable, since the bloodstream of an organism is linked to the most of its individual cells, able to transport nutrition, lipids, hormones, cell metabolites, and e.g. dead blood cells. However, Hyötyläinen and Orešič [55] reported that they found a more stable lipid profile in serum than plasma. Hence, a potential alternative for a more reliable analysis could be developed. For plasma, their review suggests differences within sample types, such as lower lipid content in citric acid containing plasma compared to EDTA modified plasma, which might influence the stability of lipids. [55]

285

286 **2.3 Profiling of metabolic alterations**

Metabolic profiling of lipids included a discrete comparison of two different diets or other similar analyses between controlled base and altered states of an organism's lipidome (*Table 2*). In profiling of homeostasis the profiling of metabolic lipids utilizes the main analysis (UHPC-MS/MS), investigates the lipid compounds, and studies the dependence and correlation between lipid species and their alterations. Equilibria between lipids in a lipidome may give insight to an organism's metabolic response to its environment. As an example, Zalloua et al. [62] noted a correlation between serum sphingomyelins (SMs) and plasma cholesterol (i.e. LDL, HDL and total cholesterol). [62] metabolite features (m/z 750-810, associated with SMs) expressed a strong correlation to cholesterol.

296 **2.4 Profiling of diseases**

When triggers concerning a specific lipidomic pathway achieve an extended time to stress the organism, an allostatic load will accumulate (early phase of a clinical condition) until the organism either recovers from the load or fails in the process. Upon failure, a breakdown of the allostatic

300 adaptation can be observed as an over- or decompensation of the metabolic pathway biomarkers; a
301 time/concentration threshold essential for clinical diagnostics [78]. Biomarkers studied between
302 2017-2019 and in the fashion of this disease model were listed in *Tables 3-4*.

303 The field of lipidomics has been widely studied for both pathological diagnostics and identification
304 of biomarkers [4]. Mass spectrometry in lipidomics enables discovery, identification, and
305 quantification of lipids in mycobacteria and applications to unravel novel functions of the
306 metabolites. The mycobacteria [81] genus is a group of acidic species characterized by a lipid-rich
307 waxy cell wall, which is much thicker than that found in most other bacteria. Mycobacteria have a
308 high lipid content and complex lipid profile including several unique classes of lipid. Notable
309 members of the genus include *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and
310 *Mycobacterium ulcerans*, causative agents of tuberculosis (TB), leprosy and Buruli ulcers,
311 respectively. In recent study, MS was used to detect mycolic acids in archeological bone samples.
312 Fourteen mycolic acids were detected in a skeleton from the Neolithic period with bone lesions
313 characteristic of skeletal TB. In this case, identification of lipids unique to *M. tuberculosis* offered
314 extra evidence of TB infection to complement diagnoses based on anatomical features and analysis
315 of bacterial DNA. [79] According to Daffe and Draper the amounts lipids in the cell of
316 mycobacteria make up 30–60% of the dry weight. They have important biological roles, notably in
317 determining virulence and drug resistance. [82] Lipids have importance in mycobacterial infections
318 and they have renaissance in these bacteria.

319 For ovarian cancer, Perrotti et al.'s [80] review on multiple large studies (n>40) strongly suggests
320 an increase of LPLs (specifically LPAs, which have been proposed as early-detection plasma
321 biomarkers) and a change in the FA profile. One explanation for LPL increase was suggested [80].
322 In addition, Zhang et al. [83] found potential biomarkers in the groups of TGs (decreased), PCs and
323 LPCs (increased). [83] The increased LPC levels caused by “deregulation of phospholipase A2”
324 mentioned by Perrotti et al. [80] Qadir and Malik also reported decreases in TG levels as well as

325 HDL-cholesterol, cholesterol and LDL-cholesterol of ovarian cancer patients [84]. Furthermore,
326 Perrotti et al. [80] agreed the research of Zhao et al. [86] that GP LPL-profiling can diagnose
327 ovarian cancer in patients [80].

328 Hu et al. [85] also listed lipidomic studies concerning epidemic disease, cancer, inflammation,
329 dysfunction, genetic disease and drug treatment. Lipidomic data on diseases can also be compared
330 with supporting results of other analyses, as is done for instance with so-called Mendelian
331 Randomization (MR) [87], [88], a statistical-epidemiological method where an risk factor's
332 (biomarker, exposure) causality to an effect (disease) is evaluated. Since genetic influences are not
333 affected by confounding factors (i.e. life choices, environment), a large population of patients can
334 be grouped according to their biomarker-associated genetic variants (i.e. variation in genetic code)
335 and compared with the frequency of a disease in that population. [88] For solid evidence of
336 causality, all the genetic variants must be associated with the biomarker that is studied. [87]

337 **3 Sampling and storage procedures**

338 Sample preparation needs specific care, since decomposition of chemically stable lipids is
339 considered mainly in the biological way [30]. Directly after isolation from organisms, self-
340 degradation of lipid compounds initiates via enzyme activation at room temperature. To avoid this,
341 proteins need to be precipitated before analysis which is usually done with methanol or isopropanol
342 [30]. Alternatively, snap freezing of samples is used for storing the authentic samples [114]. Protein
343 precipitation is utilized especially for liquid matrices for improved sensitivity in detection [115].

344 Double bonds of unsaturated fatty acids are susceptible to peroxidation, hydrolytic degradation, and
345 oxidation. Especially, after isolation and extraction, antioxidative properties of biological
346 compounds may be reduced or lost during storing. Major factors contributing to degradation are
347 formation of ice crystals combined with osmotic shock when freezing samples, which decreases the
348 volume of aqueous solution, breaks structures (e.g. cell membranes) between lipids and enzymes
349 which consequently hastens lipid breakdown. [114]

Jurowski et al. [114] reported drastic changes in lipid composition of plasma, when the sample was stored for 1-3 years at -20 °C. Furthermore, Jurowski et al. [114] observed an increase in concentrations of saturated FAs and decrease of unsaturated polyFAs. The reason for that were enzymes and activity of antioxidants. [114] Furthermore, Hyötyläinen and Orešič [55] informed that a drop in lyso-phosphatidylcholine (LPC) concentrations is detected in less than two hours (storage temperature 4 °C). In total, LPC concentrations increased within 24 hours, potentially due to activity of the phospholipid-splicing phospholipase A2. On the other hand, most PCs and sphingolipids seemed to stay stable for this time even at room temperature. [55] Thus, erythrocyte FAs in plasma are already degraded within one week at storage temperatures between 4 °C and -20 °C. Jurowski et al. [114] informed that samples stored at -60 °C do not change in one year of storing. [114] Hyötyläinen and Orešič [55] agreed the stability of lipid containing samples stored at -80 °C for at least six months, but a change was seen at a time span of five years. [55] Sampling, sample storage and pre-analysis lipid degradation has been discussed in greater detail by Hyötyläinen and Orešič [55], Patterson et al. [115], Tumanov et al. [116], Jurowski et al. [114] and Monnin et al. [30].

4 Sample preparation for lipid analyses

The main limitations of lipidomics analysis are the chemical complexity of the lipids, the range of concentrations at which they exist, and the variety of samples usually analyzed. [117] Especially, sample preparation is needed to prevent lipids at high concentration to suppress ionization of low concentrated lipids in mass spectrometry detection.

Here, only few examples are discussed to show the complexity of the matrices, when HPLC-MS methods are used for information collection of lipids. Altered lipid metabolism plays a key role in the pathogenesis of common diseases. Most of the bioactive mediators originate from the cleavage of lipid constituents of cellular membranes under the activity of phospholipases. In amniotic fluid research, lipids identified were used as indicators for stages of pregnancy or pregnancy

375 complications. [5] Total lipid and phospholipid concentrations were observed to increase from 24
376 weeks of gestation until labour. Thus, routine tests for fetal development evaluation of
377 lecithin/sphingomyelin-ratios could be used for long-term monitoring. Furthermore, lipidome
378 profiles of amniotic fluids were observed to be different between infants born in term and those of
379 pre-term children. [5]

380 Primary ascites sample matrices have discovered to have abnormal formation of intra-peritoneal
381 fluid in a patient's abdominal cavity. [118] Two types of lipid samples could be used according to
382 formation: non-inflammation induced (e.g. hydrostats- or osmosis-produced) transudates matrix and
383 inflammation-accompanied substances secreted by an organism. Transudates are suspect of de-
384 compensation of blood circulation, kidneys, and liver, whereas exudates are formed in oozing of
385 ruptured or otherwise damaged cell tissues formed in complications of cysts in ovarian cancer
386 tumors. In contrast to commonly clear transudates, in intraperitoneal space exudates are noticed to
387 have higher protein-concentrations due to the less filtrated nature. [118]

388 In the Folch-method [119] about 5% of proteins are left in the organic phase, when lipids are
389 isolated. That may contribute to matrix effects and higher background noise. [115] In comparison to
390 the Folch method, BUME method [120] is an extraction technique considered superior from the
391 environmental, economic and preparative standpoints, since extraction needs no chloroform and
392 needs less solvent. The method allows to extract all matrices into millilitre volumes with
393 comparatively lowered safety hazards. [121]-[124]

394 For samples with low lipid concentrations, solid phase extraction (SPE) [58], [125] or lyophilization
395 (i.e. freeze-drying) [125] can be considered to concentrate lipids in a sample for the analysis. Teo et
396 al. [125] introduced a concept of energy-based extraction methods (i.e. pressurized liquid,
397 microwave- and ultrasonic-assisted extraction) alongside polarity-based extraction methods (i.e.
398 single solvent, liquid-liquid, solid phase extraction combined with supercritical fluid extraction
399 which was developed by Jurovski et al. [114] The latter techniques are used more frequently in

lipidomics studies. [125] Energy-based techniques warm the extraction system to achieve a faster and more efficient removal of lipids from the matrices. [125] *Table 5* lists extraction and sample purification methods used in lipidomics.

Bang et al. [127] conducted solid-phase extraction (SPE) in a superabsorbent polymer (SAP) device for small sample sizes to minimize carry-over of aqueous solvent. In the procedure water was gelated with the SAP polymer, and organic solvent was adsorbed and collected lipids with recoveries similar or greater than in the Folch extraction [127]. Other extraction methods [58], [114], [119]-[125] as well as single solvent and liquid-liquid extractions with acetonitrile or chloroform-methanol 2:1 (v/v) [51], [128] or with methyl-tert-butylether (MTBE, i.e. Matyash) extraction [55], QuEChERS methodology (Quick, Easy, Cheap, Effective, Rugged, and Safe) [127], and the “Bligh and Dyer” method with chloroform-methanol [129]. The use of a chloroform-methanol solution for lipids extraction was observed to isolate all major lipid classes, which were enriched in the medium polar chloroform phase. [126] The use of MTBE as the solvent led to higher extraction efficiency for unsaturated fatty acids, GPs and Cer, while chloroform-methanol mixture favoured the isolation of saturated fatty acids. [117] However, the recommended sample preparation for isolation of polar lipids in adipose tissue should be combination of liquid–liquid extraction and an SPE to enhance detection of GPs in MS.

5 Liquid chromatography in lipidomics

Some analytical techniques are specifically better used to identify lipid classes and sample types than individual lipids, why prior knowledge about targeted analysis is to choose the suitable lipid method. According to Hu et al. [85], NMR techniques can identify and quantify (isolated) lipid fractions as well as provide lipid-associated disease profiles. However, NMR can easily falter in the face of complex sample matrices and their analysis. [85] Apart from desorption and probe MS techniques, imaging techniques lack selectivity for lipid species analysis (though they do have the advantage of nonspecific lipid localization). [132]

425 **5.1 High-performance liquid chromatography of lipids**

426 High-performance liquid chromatography (HPLC) is a method using a liquid mobile phase and
427 solid stationary phase. Analytes are separated depending on the strength of their interaction between
428 stationary phase and mobile phase. Reversed phase liquid chromatography (RP-LC) dominates the
429 chromatography in lipid research and, for that reason, mostly nonpolar and medium-polar lipids are
430 studied. In RP-LC most of the lipid species and internal standards elute at different times, thus
431 experience different matrix effects and different solvent composition, which influences their
432 ionization and may result in inaccurate quantification. [133] However, recent studies with normal
433 phase (NP) stationary packings and with the newest technology in hydrophilic interaction
434 chromatography (HILIC) provides lipid class-specific separation. This has great advantages in
435 terms of quantification, since usually analytes and internal standards show similar retentions. [134]
436 The ultimate advantage is that identification of lipid species with lipid classes is straightforward.
437 Especially, HILIC separation has gained active development recently, since the method separated
438 lipids by distribution, adsorption, ion exchange, and exclusion chromatography. [135] The literature
439 about lipids research with HILIC have slightly increased during the recent years.

440 Since there is no separation based on the chain lengths of FAs in normal phase chromatography
441 separation, which is the phenomenon in reverse procedures in RP-LC, the precursors affect the
442 retention in the NP column and identification of their MS fragmentation patterns. Full scan MS
443 spectra are usually collected either in negative ionization or in positive ionization mode. Data
444 dependent tandem-MS (MS^2) was performed in parallel with ESI-Orbitrap-MS scanning on a
445 Fusion mass spectrometer, which is accurate enough for lipid class confirmation. [141] However,
446 identification of chain lengths and saturation states of lipids need high resolution MS
447 instrumentation, when ion resolution is expected further to improve separation efficiency obtained
448 in LC. That was the reason in the study by Jeucken et al. [70], who used a HILIC procedure to
449 observe retention of inorganic ions in lipid matrices. Inorganic ions lead to formation of distorted

lipid adducts in identification with MS. In addition, inorganic salts are shown to decrease repeatability of quantitation, but they are known to co-elute with lipids. [136] Typically, additives are used, since they are dissolved into water or into water-organic solvent mixtures. They allow stabilization of adduct ions concentrations. Additives may form complexes with neutral species due to adduct formation during MS ionization. In lipid analyses, additives have shown to quench and suppress counter-ion species. Furthermore, they show to control the formation of predictable adduct ions, when weaker ligands are present or concentrations of the main ligands are negligible. Moreover, as Erngren et al. [136] have demonstrated with positive mode ionization, inorganic ions improve separation in LC, since the ions are retained onto a normal phase separation column material (such as in HILIC), and they are not co-eluted nor form complex adducts or clusters, thus resulting in decreased repeatability in quantification. [136] This was also the reason for using additives in post-column derivatization by Monnin et al. [30] They compared their LC method performance by using acetic acid and ammonium hydroxide as the additives in the eluent, when detection was done with negative ionization MS. Generally, the study showed that additive ions could form pseudo-neutral adducts with charged lipids by utilization of interaction with the stationary phase via adduct-driven polarity manipulation.

5.2 UHPLC systems in lipid analyses

Ultra-high-performance liquid chromatography (UHPLC) was introduced to use fast chromatography with nano-columns [131] Fekete et al. [138] showed that good separation efficiency of lipids at abnormal slow eluent flow through less than 2 μm sized particles was possible. The success of the discovery was followed by a near exponential growth of UHPLC and UHPLC-MS publications in the following ten years. Based on Danne-Rasche et al. [140] a Venn diagram is a useful tool to visualize similarities between data from normal and ultra-high LC systems. They discovered that UHPLC and capillary-packed nano-LC (contrary to conventional LC) is necessary for the detection of over half of yeast's lipidome.

475 Jeucken et al. [70] used UHPLC to separate HeLa-cell lipid metabolites. In their one-step protocol,
476 proteins and other macromolecules were removed and the protein-free chloroform-methanol
477 mixture was injected directly to LC-MS. The extracted lipids were loaded onto a HILIC column
478 [141] During a run time of only 4 min, they achieved separations based on end-groups of lipids
479 (*Figure 6*). In the study sample preparation of HeLa cells [70] was done on a 96-well plate, on
480 which the cells were filled with the cells and cultivated in the presence and absence of lipid
481 metabolic inhibitors. The studies were done to observe changes in lipidome expression, when the
482 process was altered at the metabolic state.

483 **5 Mass spectrometry in lipidomics**

484 The first reports of mass spectrometric (MS) analyses of complex lipid mixtures via soft ionization
485 techniques (matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI))
486 were developed in the 1990s. [142] In particular, the use of atmospheric pressure ionization
487 (especially electrospray ionization, ESI) and the coupling of liquid chromatography (LC) to MS
488 have allowed the identification and quantitation of ever-increasing numbers of lipid species. Han
489 and Gross [142] showed with ESI-MS that they could make structural determination and even
490 quantitative analyses of individual phospholipid molecular species at sub-picomole level from
491 chloroform extracts of biological samples. In lipidomics mass spectrometry is the only reliable tool
492 for structural identification. Nevertheless, lipidomics is challenging with the many compounds,
493 which cannot yet be expressed as fully as the compounds in genomics and proteomics, which are
494 well represented in various forms at leading research institutions worldwide. [49]

495 **6.1 Mass spectrometric analyses of lipids**

496 Lipid analyses from simple profiling to complex metabolite interaction mapping is primarily made
497 with various kinds of ionization techniques in mass spectrometry. Holcapek et al. [143] divided
498 mass spectrometry into three major approaches in lipidomics: direct infusion MS (DIMS, i.e.

499 shotgun lipidomics, flow injection MS), chromatographic methods on-line coupled with MS, and
500 desorption techniques in MS. [143], [144]

501 DIMS was recommended to be used as a fast-diagnostic method. [186] The direct sample infusion
502 method to MS analyses uses one sample per one run at the time. Thus, it is a very appealing method
503 with multiple precursors and fragments. With DIMS, very high ion resolution and resolving power
504 are needed. Because of many fragment ions continuously present lipid samples are diluted before
505 injection to MS to avoid detector saturation. Furthermore, knowledge on spectral interference is
506 good to be available for quantitation data and considering identification of similar compounds, like
507 identical isomer fragments. [144] Furthermore, matrix effects may play a large role in analyses.
508 [146] Anyhow, desorption techniques are ideal mass-spectrometric imaging of tissues and cells.
509 [143] When locations of the origin of lipids are not possible in conventional LC-MS, desorption
510 DIMS offers a fast solution. For this localization of lipids, multiple approaches have been attempted
511 such as mass spectrometric imaging (MSI) [111], [143], [144], [147] liquid extraction surface
512 analysis (LESA) [140], [148] and probe research [147]-[149]

513 Solvents are very important in MS analyses of lipids (Monnin et al. [30]). Ammonium hydroxide –
514 acetic acid composition were studied in aqueous solution to optimize degree of ionization of lipids
515 in negative ESI-MS. The tests were made for lipids in the classes PA, PE, PC, LPC, LSM, Cer,
516 LPA, and PS. [30] Usually, in positive ESI-MS water-methanol and water–acetonitrile solutions are
517 used with modification of formic acid or acetic acid. [150] Lorenzen et al. used water, acetonitrile,
518 and isopropanol as the eluents in UHPLC-MS/MS studies and identification with both negative and
519 positive electrospray ionization modes. The eluents (pH 5.5) contained also ammonium acetate and
520 formic acid as a buffering additives in order to maintain the lipids in the same ionic state during the
521 separation process. [151] To gain all metabolites of a subclass, Griffiths and Wang [188] single-
522 extract STs with ethanol to acquire oxysterols esterified with sulphuric acid and linked sugars. [188]

Table 6 lists examples of lipid classes and molecular species formed during electrospray ionization in negative ESI-MS mode. The fragments used for lipid class identification were $[M + H]^+$, $[M + Na]^+$, $[M + NH_4]^+$, $[M - H_2O + H]^+$ in the positive mode and $[M - H]^-$, $[M + HCOO]^-$, and $[M + CH_3COO]^-$ in the negative mode. [152] Cajka et al. [152] noticed in their project that PEs were the only detectable glycerophospholipid species, which were distinguished by the $[M - 141 + H]^+$ MS^2 fragment resulting from the neutral loss of the polar end-group. Isolation of lipids from cells was made according to Bligh and Dyer [129] and with reverse-phase liquid chromatography for sample introduction to MS.

6 Method optimization protocols

For understanding a metabolic-lipidomic system, the lipidome or the relevant lipid group should be mapped out. Merrill Jr. et al. [153] designed a workflow for sphingolipid (SP) analyses of a lyophilized cell culture by using multiple LC-MS/MS protocols for specific subclasses. [153] However, performing comprehensive analyses of lipid polarity ranges is still not sought for until today.

To help method optimization, there are four protocols that inform distinct target groups in analyses. They deal with (1) the extraction procedure for the preparation of sphingolipids for analysis by LC MS/MS, (2) reverse-phase LC, (3) normal-phase LC, and (4) the order of steps to follow in setting up an LC-MS/MS for a new biological sample. Furthermore, they mentioned the relation between sphingolipids (SPs) and their metabolites, which remind the biochemical equilibrium present in a biological system.

6.1 Lipid classes studied with liquid chromatography

Teo et al. [125] studied various biofluids and tissues that were analyzed with different chromatographic and ionization methods with mass spectrometry. LC-MS is necessary, when comprehensive screening of biomarkers is required (e.g. in cancer research[137]). Primarily, GLs, GPs, SPs, and STs in liquid-type biological materials (e.g. blood and secretions) and solid tissues

548 (e.g. eyeball, fibroblasts and skin) were studied with LC-MS. [125] Later, Jurovski et al. [114]
549 showed that FAs and CEs are also important lipids to study clinical samples, when there is a need to
550 identify the lipid profiles.

551 Since variation of lipid classes is enormous, it is not surprising that the polarity of lipid species
552 varies a lot. Figure 7 demonstrates the lipid-subclass ranges of four distinct chromatographic
553 approaches, namely lipids separated in reverse phase and normal phase LC separation strategy. The
554 subclasses include to analyse separately both nonpolar and polar lipids with liquid chromatography
555 separation. Based on the structural differences, the lyso-forms are more polar than their
556 counterparts, which have an extra fatty acid chain in the structure. Particularly, polar and mid-polar
557 (PS, PG, PI, Cer) lipids seem to be species that are often identified with negative mode in MS,
558 though more species are primarily found with positive mode identification. As to the GLs groups,
559 only MG has a fatty acid chain that is small enough to be rather polar. As a thumb rule, SPs are
560 mid-polar lipids, since they are amines and have nitrogen-bound fatty acid chains.

561 **7 Lipids important in clinical and medical samples**

562 Lipids have important roles in the profiling of cell types (e.g. cancer cell lines or bacteria [114]) as
563 well as directly from various biological fluids and biotargets (e.g. plasma [45], [112] and human
564 amniotic membrane [154]). When cells are the examined matrices, they are usually harvested by
565 ultracentrifugation, washed with water, resuspended, incubated, extraction phase isolated with
566 centrifugation, re-extracted, filtered, dried under nitrogen, and dissolved in methanol-
567 dichloromethane-water mixture (35:70:5, v/v/v) [151], though sample preparation methods vary.
568 *Tables 7, 8, and 9* list samples analyzed between 2017 - 2019 in lipidomic UHPLC/MS research.
569 Cell lipids are important biomolecules for diseases due to metabolism.

570 The range of unconventional biological extracts and tissues, as described by Jurovski et al. [114], is
571 very broad. Furthermore, *in vitro* biological cell culture analyses reached a high variation of studies

among themselves, which lead to include them separately in *Table 9*. The multi-matrix approach was a pool of plasma, urine and faecal matter of the same patient. [155]

Tables 10 and 11 list all lipids in the articles where identification of various lipid groups was discussed. Just like fatty acyls (FACyls, including FAs, BMPs and CLs) were reported for physiological profiling [50], [53], [58], [107], lipido-metabolomic [67], [69], [70], and disease biomarker studies [5], [9], [95], [98]-[100], [102], CEs were frequently found in method development [46], [108], [130], [155], [189] and cancer research. [46], [108], [110], [113] Also, sub-groups, the totality of identified lipids, and biomarker lipids were included. In total, a range of 5-700 total lipids and 5-87 proposed biomarkers were discussed in the articles. Typical GLs in articles included DGs and TGs, with MGs or BMP. [69]

Profiling lipid metabolites of hepatocellular carcinoma revealed down-regulation of TGs having less than two double-bonds in their structures, which was also observed in ceramides. Conversely, up-regulation was noticed for only GPs and SM. Particularly, new information on TG, PC, PE, and PI trends was uncovered. [111] Lipid profiles differ a lot from sample to sample, though some consistencies remain. During negative mode ESI-ionization, Tumanov et al. [116] claimed to have observed a bias in LPA quantitation. Noteworthy is that the concentration of LPAs may be easily overestimated due to typical over-abundance of LPCs combined with the choline moiety which is lost during ionization process in MS. [116]

9 Metabolite identification with MS and data analysis

The experiments with both positive and negative ionization modes in MS are useful in lipid analyses, since the data can be combined in order to identify the maximum possible number of lipids. [151] Usually, lipid standards are needed in order to evaluate the chromatographic profiles and fragmentation behavior of lipid species. Comprehensive characterization and quantification of molecular lipid species was shown to be achieved by spiking the lipid extracts with unique lipid

standards and using selective ionization conditions for sample infusion to have structure-specific mass analyses by mass spectrometry.

Liebisch et al. [39] proposes two ways for solving the lack in identification: Either mass accuracy must be increased to separate two nearly identical species or fragmentation of MS^n must be developed. Thus, fragmentation behaviour of PI-Cers (GIPC-Cer) have been described previously and structures were characterized to have a polar head group, fatty acid, and sphingoid base [151], [162], [163]. Ceramide molecules form distinct domains in the cell membrane, which may serve to re-organize cellular receptors and signalling molecules [164]. ESI-MS data for identification and characterization of the structure of PGs, PEs, CLs, and glycerolipids (DGs and TGs) are also available. [164]-[168] The presence of PGs, PEs, and C_{26} -dihydro Ceramide (Cers), as well as the presence of distinctive α -hydroxylated FAs together from complete FA profiles of strains have also been published. [169]-[172]

When lipids were studied with UPLC-ESI-MS with eluents containing ammonium acetate and formic acid, PGs, PEs, and CLs were detected as $[M-H]^-$ ions, and Cers being both hydroxylated and no hydroxylated as $[M+HCOO]^-$ ions in negative ion mode. In positive ionization mode PEs, Cers, MGs, DGs, and TGs could also be detected, but the former two were detected as $[M+H]^+$ ions and the latter two as $[M+NH_4]^+$ ions. [151], [173]

Research on routine *in vivo* analyses of samples included six categories of matrices, primarily blood (plasma, serum, erythrocytes and blood platelets), faecal matter, and urine. One of the revised papers even proposed a multimatrix analysis by pooling plasma, faecal matter, and urine in one sample to increase method repeatability and lipid coverage. [156] Unconventional biological materials, i.e. harder-to-get as *in vivo* samples of 18 different biofluid and tissue types, of which the most frequent topic of interest was liver tissue. [15], [63], [111] [127], [155], [160] In addition, experiments with 18 different cell lines were found to be cultured *in vitro*.

Moreover, a new way for improved identification and quantitation in lipidomic studies was found: The utilization of the lipidome isotope labelling of yeast (LILY) standard to produce (non-radioactive) isotopically labelled eukaryotic lipid standards in yeast for normalization and quantification in mass spectrometry. [174] When looking at analytes of low concentrations, higher mass accuracy is often preferred to exchange lower resolution and to increase sensitivity. A slight trend of multiple studies on big lipid structures (e.g. cell cultures, exosomes, [98], [110] lipid droplets, [60] and liposomes [159]) can be observed. Partially, this can be explained with new trending asymmetric flow field flow fractionation (AF4) [96], [97], [110] instruments, which has ability to separate precisely biomolecules by mass into smaller fractions before primary analysis. In UHPLC-MS lipidomics, the use of a pooled quality control (QC) sample in the analysis sequence has been standardized, which mean that according to the literature search in the year 2017 11 papers from 25, in 2018 14 papers from 22, and in 2019 8 papers from 10 showed the trend of increased pooled sample usage (even in qualitative analyses). [150]

10 Untargeted vs targeted approaches

It is currently not possible to comprehensively measure the lipidome of a cell or tissue in a single experiment. Either the precise alteration in lipids to expect is not known in any given case. Thus, the first studies are often exploratory, which means that they are done using untargeted profile monitoring. Thus, the MS methods need high mass accuracy and resolution. Analyses of fragmentation pathways has led to a detailed understanding of the connections between different building blocks found in lipids (such as fatty acids, sphingoid bases, and head groups. Precursor lipids are determined based on characteristic fragment ions, as seen in *Figure 8*. Other targeted approaches based on tandem mass spectrometry are now available for analysis of many different classes of lipids and in complex mixtures. [175], [176]

Lipids have some unique advantages and challenges for mass spectrometric analyses. The application of electrospray ionization to crude lipid extracts without prior fractionation is one such

example, as it has perhaps been more successfully applied in lipidomics than in any other discipline. The diverse molecular structure of lipids means that collision-induced dissociation alone may be limited in providing unique descriptions of complex lipid structures. [176] [177] A conventional targeted approach needs a lot of calibration standards in lipidomics. [50] It is not feasible to calibrate all lipids individually. Thus, a simpler compromise on class-representative lipid standards with optimally chemical similarities are often applied in lipidomic studies. Ideally, however, standards and analytes should be chemically equivalent. Though expensive, the most used internal standards are deuterated standards, most often by Avanti Polar Lipids (Alabaster, AL, USA). Typically, the protons are deuterated at the FA end of the carbon chain with either 7 or 9 deuterium atoms. For representative standards in analysis, a self-made or commercial standard mixture is typically used (e.g. a SPLASH mix [130] or well-known organic standard [porcine brain, chicken egg, e. coli] [161]). The molecular ion and its fragments that still include the deuterated FA chain experience a noticeable shift in their m/z value compared to their non-deuterated counterparts. Since fragmentation is identical for both variants, this m/z differentiation is ideal for ISTD normalization via EICs. [174]

660

661 **11 Current statistical methods used in mass spectrometry**

Targeted and untargeted methods are two distinctively different approaches, as well as the means of data processing. Targeted data processing of metabolites was divided to five phases: (1) the acquisition of raw data, (2) the contemplation of which database should be used when considering the research question and analytes, (3) the pre-processing and identification of these metabolites, (4) the normalization and quantification of the identified species, and (5) the reflection of the results on the biochemical/physiological context of metabolic pathways [179]. In contrast, untargeted approaches need more careful experimental MS data and more data modifications, but also pre-processing for detection. In addition, resolution in screening and identification of the relevant

670 biomarkers from the totality of features/peaks need to be considered, although the non-targeted
671 steps include the same pathway elucidation as in the targeted analyses [179].

672 In lipidomic research, statistical methods have enlarged into a broad variety of numerical tests and
673 visualization techniques. Typically, statistical methods include a null hypothesis to test similarities
674 by using one dataset. However, the p-value parameter in the Student's t-test, [56], [57], [64], [65],
675 [67]-[69], [72], [73], [75], [76], [92], [97], [98], [94], [102], [104], [105], [127], [156], [159] the
676 non-parametric Mann-Whitney U-test, [56], [74], [76], [93], [99], [103], [104]-[106], and the
677 analysis of variance test (ANOVA) [63]-[65], [98], [107], [109], [113], [156], [157] are for
678 comparison of two or more datasets. These methods are often used for validation of analytical data
679 and for detection of changes between controls and authentic samples. Correction methods such as
680 the Benjamini-Hochberg test [180] are used to calculate the false discovery rate (FDR), i.e.
681 minimizing false positive data from the dataset. [74], [75], [76], [105], [106], [181] An unequal
682 variance test like the Storey [74], [182] or Welch [87] t-test may also be used. [74], [87]

683 The Mann-Whitney U-test [183] is used in mathematical data handling, when all parameters are
684 variables to evaluate similarities of two independent dataset medians with a null hypothesis. For
685 two sets with same or different sample sizes, the values of both datasets are sorted from the smallest
686 to the largest ones. A Mann-Whitney U-test can be fitted for non-Gaussian distribution data. It is
687 typically combined with a Benjamini-Hochberg (or Bonferroni-Holm [69]) test to exclude false
688 positive values, thus giving the false discovery rate (FDR). These methods were applied in multiple
689 studies to limit uncertainty in the results of lipids. Furthermore, Paepe et al. [156] and Gong et
690 al.[105] used cross-validated ANOVA test (CV-ANOVA) to improve reliability of the identified
691 analytes.

692 Lately, Tietz-Bogert et al. [93] calculated the FDR value in a lipido-metabolomic study by
693 searching significant biomarkers of primary sclerosing cholangitis, [93] which is a disorder of lipids
694 in the bile duct of unknown origin. The concentrations of lipid species in control samples of healthy

695 individual's and the clinical samples of sick patient's blood and bile were compared to find changes
696 with a statistical significance of 0.01. [93]

697 Machine learning and software advancements for multi-ionic identification have allowed to
698 evaluate MS spectra produced with UHPLC-MS *in silico*, which is only demonstrated in a few
699 recent articles. [47], [57], [90], [113], [161] Due to machine learning techniques and their
700 discovered use in omics, automated lipidomic analyses, ROC/AUC cross-validation analyses, [90],
701 [105], [181] random forest studies, neural network applications, [184] *in silico* spectra evaluation
702 [161], and CCS value [47] generation algorithms have become in use. More detailed information is
703 available in ref. [132].

704 LipidBlast methodology has been created for generating MS/MS spectra (Figures 9 and 10). Lipid
705 analysis of polar lipids can be performed with tandem mass spectrometry and mass spectral library
706 search. LipidBlast was mostly developed with ion trap tandem mass spectra, but it can be used with
707 data from other platforms such as QTOF mass spectrometers.

708 The LipidBlast library works with low-resolution and high-resolution instruments. [185] An m/z
709 precursor-ion filter first filtered the data, and a subsequent product-ion match generated a library hit
710 score that reflects the level of confidence for compound annotation. [185] It must be noted that a
711 "closest-match" bias can be apparent when identifying an analyte with a library search. [187], [188]
712 As the library may be incomplete, possible isomers must be considered when validating data.

713 **12 Discussion**

714 Lipid function on the tissue-level is rarely explained via cell-level mechanisms, which are the only
715 frequent mechanisms mentioned being lipid signaling. In speculations, lipids may be distributed
716 between tissue cells via vesicles or other means of expulsion depending on the tissue. Do the well-
717 established tissue types (epithelium, muscle, connective/protective and nervous tissue) have inter-
718 or intra-class uniformity or variations in lipid function, which could be supported by cell-level
719 observations?

720 Due to the structural complexity of lipids, their quantification is not easy to perform. Different
721 strategies and techniques should be addressed depending on the targeted analytes. The difference of
722 ionization efficiency between lipid species, depending on their structure, is indeed a real limitation
723 of this technique.

724 An increasing number of studies report about poor quality of lipidomics data with misidentification
725 and inaccurate or inappropriate quantification of lipid molecules. These studies primarily use
726 standardless [145] and untargeted metabolomics approaches. [170] To all intents and purposes, the
727 reasons for the poor data quality seem to be analytical, bioinformatics, and educational aspects.
728 Therefore, it is necessary to implement reporting standards for lipidomics data to share with the
729 scientific community [39], [134]. These standards need to cover both shotgun and HPLC–MS
730 approaches. Only applying both approaches in a complementary and confirmatory way permits a
731 comprehensive and accurate coverage of the lipidome, as earlier was documented by G. Liebisch
732 [134].

733 Plasma membrane asymmetry of two phospholipid leaflets could have implications for membrane
734 potential as well as phase properties (e.g. signalling, diffusion and biochemical activity). Possible
735 mechanisms, such as change in lipid composition or shift in leaflet asymmetry by local synthesis or
736 lipid transportation could be an interesting subject for lipidomic research. Evidently, in real time
737 this would need improvements in the selectivity of in situ lipid imaging techniques, which may
738 possibly be desorption MS [111], [143], [144], [147], or Raman [190], [60], [150] technology in the
739 future.

740 Some interpretation of the data in the low-abundance field may lead to over speculation. Han [16]
741 proposes small amounts of lipids to be originated from leftover reactants of imperfect biochemical
742 reactions, since virtually no perfect thermodynamic conversion from precursors to products exists.
743 For example, when LPE and LPC is observed in a matrix, PA-derived GP species must be present
744 from de novo plasmalogen synthesis. [16]

Antonny et al. [12] reminds that FA chain function of lipid classes is less understood, which would insinuate more separation by reversed-phase chromatography. Traditionally, nonpolar and medium-polar lipids are studied with non-polar phases and two-component mobile phases made of water or buffered water and organic solvents in RP-HPLC. The advantage of ternary mobile phase compositions made of medium polar organic solvents, water or buffered water, and methanol or acetonitrile mixtures would be needed to separate and elute also polar lipids. In addition, the possibility of sequentially performed NP-HPLC and RP-HPLC coupled with MS should be more studied for lipids in biological samples. On the other hand, Liebisch et al. [39] promotes NP-HPLC and HILIC quantification over RP-HPLC for providing polar group selectivity by class selective separation and, thus, internal standard co-elution. [39]

13 Conclusions

The paper shows the fact that new methods for lipids and applications in clinical and medical research are still challenging in life science. To obtaining a truly complete overview of all lipids in a sample has remained very challenging due to their enormous structural diversity. A combination of sample extraction and separation procedures is required. Given the central role of lipids as key metabolites with remarkably diverse biological roles, the field of lipidomics may follow a trajectory comparable to the developments seen in genomics and proteomics over the past decade.

This review compiles recent lipidomic studies from the angle of UHPLC-MS research from the past three years (2017-2019). Identified lipids by subclass, biomarkers, clinical and in vitro sample matrices (i.e. biological materials) as well as specified topics on homeostatic, metabolic and disease-metabolic profiling are represented alongside quintessential analytical, biological and chemical insight for data interpretation. As an example, cell tissue data in research has rarely been put into context with cell-level observations. So far, signaling is seen as an only connection between these levels of biological organization.

14 Acknowledgements

We want to thank the Institute of Analytical Chemistry at the Johannes Kepler University Linz for the support in effort to understand lipidomic research. Mr Joel Tiitinen is also acknowledged for drawing the graphical abstract.

Funding: This research received no external funding.

Conflicts of Interest: All authors declare no conflict of interest.

15 References

- [1] Serhan, C. N., & Savill, J. (2005). Resolution of inflammation: the beginning programs the end. *Nature immunology*, 6(12), 1191.
- [2] Ståhlman, M., Ejlsing, C. S., Tarasov, K., Perman, J., Borén, J., & Ekroos, K. (2009). High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry. *Journal of Chromatography B*, 877(26), 2664-2672.
- [3] Koriem, K. M. M. (2017). A lipidomic concept in infectious diseases. *Asian Pacific Journal of Tropical Biomedicine*, 7(3), 265-274.
- [4] LIPID MAPS: Tutorials and Lectures on Lipids, Categories of Lipids. 02.10.2018 http://lipidmaps.org/resources/tutorials/lipid_tutorial.php#FA
- [5] Ghosh, A., & Nishtala, K. (2017). Biofluid lipidome: a source for potential diagnostic biomarkers. *Clinical and translational medicine*, 6(1), 22.
- [6] Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R., Shimizu, T., Spener, F., van Meer, G. , Wakelam, M. J. O., & Dennis, E. A. (2009). Update of the LIPID MAPS comprehensive classification system for lipids. *Journal of lipid research*, 50(Supplement), 9-14.
- [7] Ribeiro, M. A., Murgu, M., de Moraes Silva, V., Sawaya, A. C., Ribeiro, L. F., Justi, A., & Meurer, E. C. (2017). The screening of organic matter in mineral and tap water by UHPLC-HRMS. *Talanta*, 174, 581-586.

- 795 [8] Rombouts, C., De Spiegeleer, M., Van Meulebroek, L., De Vos, W. H., & Vanhaecke, L.
796 (2019). Validated comprehensive metabolomics and lipidomics analysis of colon tissue and cell
797 lines. *Analytica Chimica Acta*.
- 798 [9] Schlotterbeck, J., Chatterjee, M., Gawaz, M., & Lämmerhofer, M. (2019). Comprehensive
799 MS/MS profiling by UHPLC-ESI-QTOF-MS/MS using SWATH data-independent acquisition for
800 the study of platelet lipidomes in coronary artery disease. *Analytica chimica acta*, 1046, 1-15.
- 801 [10] Taylor, N., White, T., & Viant, M. (2017). Defining the baseline and oxidant perturbed
802 lipidomic profiles of daphnia magna. *Metabolites*, 7(1), 11.
- 803 [11] Van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where they are
804 and how they behave. *Nature reviews Molecular cell biology*, 9(2), 112.
- 805 [12] Antonny, B., Vanni, S., Shindou, H., & Ferreira, T. (2015). From zero to six double bonds:
806 phospholipid unsaturation and organelle function. *Trends in cell biology*, 25(7), 427-436.
- 807 [13] Gibellini, F., & Smith, T. K. (2010). The Kennedy pathway—de novo synthesis of
808 phosphatidylethanolamine and phosphatidylcholine. *IUBMB life*, 62(6), 414-428.
- 809 [14] Wang, L., Shen, W., Kazachkov, M., Chen, G., Chen, Q., Carlsson, A. S., ... & Zou, J. (2012). Metabolic
810 interactions between the Lands cycle and the Kennedy pathway of glycerolipid synthesis in Arabidopsis
811 developing seeds. *The Plant Cell*, 24(11), 4652-4669.
- 812 [15] Manni, M. M., Sot, J., Arretxe, E., Gil-Redondo, R., Falcón-Pérez, J. M., Balgoma, D., Alonso,
813 C., Goñi, F. M., & Alonso, A. (2018). The fatty acids of sphingomyelins and ceramides in
814 mammalian tissues and cultured cells: Biophysical and physiological implications. *Chemistry and*
815 *physics of lipids*, 217, 29-34.
- 816 [16] Han, X. (2016). Lipidomics for studying metabolism. *Nature Reviews Endocrinology*, 12(11),
817 668.
- 818 [17] LIPID MAPS, Databases: “Classification-based search”, Lipid Categories, 22.11.2018,
819 <http://lipidmaps.org/resources/databases/index.php>

- 820 [18] Dennis, EA: "LIPID MAPS Lipid Metabolics Tutorial, Fatty Acid Biosynthesis", Powerpoint,
821 2010, University of California, San Diego
- 822 [19] Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983). Fatty acid synthesis and its regulation.
823 *Annual review of biochemistry*, 52(1), 537-579.
- 824 [20] Nelson DL, Cox MM (2005), Lehninger Principles of Biochemistry, 4th ed., W.H. Freeman &
825 Co.
- 826 [21] Manzo-Fontes, A., Slenter, D., Willighagen, E., Kutmon, M., Cirilla, E., "Glycerophospholipid
827 Biosynthetic Pathway (Homo sapiens)" *WikiPathways*, 1.11.2019, link checked 6.11.2019,
828 <https://www.wikipathways.org/index.php/Pathway:WP2533>
- 829 [22] Nagan, N., & Zoeller, R. A. (2001). Plasmalogens: biosynthesis and functions. *Progress in*
830 *lipid research*, 40(3), 199-229.
- 831 [23] Detopoulou, P., Nomikos, T., Fragopoulou, E., Antonopoulou, S., Kotroyiannis, I.,
832 Vassiliadou, C., ... & Stefanadis, C. (2009). Platelet activating factor (PAF) and activity of its
833 biosynthetic and catabolic enzymes in blood and leukocytes of male patients with newly diagnosed
834 heart failure. *Clinical biochemistry*, 42(1-2), 44-49.
- 835 [24] Kita, Y., Tokuoka, S. M., & Shimizu, T. (2017). Mediator lipidomics by liquid
- 836 [25] Scherer, M., Schmitz, G., & Liebisch, G. (2009). High-throughput analysis of sphingosine 1-
837 phosphate, sphinganine 1-phosphate, and lysophosphatidic acid in plasma samples by liquid
838 chromatography–tandem mass spectrometry. *Clinical chemistry*, 55(6), 1218-1222.
- 839 [26] Pulkoski-Gross, Michael J., Donaldson, J. C., Obeid, L. M., "Sphingosine-1-phosphate
840 metabolism: A structural perspective." *Critical reviews in biochemistry and molecular biology* 50.4
841 (2015): 298-313.
- 842 [27] Haynes, C. A., Allegood, J. C., Park, H., & Sullards, M. C. (2009). Sphingolipidomics:
843 methods for the comprehensive analysis of sphingolipids. *Journal of Chromatography B*, 877(26),
844 2696-2708.

- 845 [28] Lydic, T. A., & Goo, Y. H. (2018). Lipidomics unveils the complexity of the lipidome in
 846 metabolic diseases. *Clinical and translational medicine*, 7(1), 4.
- 847 [29] Sahu, S. K., Hannun, Y. A., & Yao, N. (2019). Emergence of membrane sphingolipids as a
 848 potential therapeutic target. *Biochimie*.
 849 chromatography-tandem mass spectrometry. *Biochimica et Biophysica Acta (BBA)-Molecular and*
 850 *Cell Biology of Lipids*, 1862(8), 777-781.
- 851 [30] Monnin, C., Ramrup, P., Daigle- Young, C., & Vuckovic, D. (2018). Improving negative
 852 liquid chromatography/electrospray ionization mass spectrometry lipidomic analysis of human
 853 plasma using acetic acid as a mobile- phase additive. *Rapid Communications in Mass*
 854 *Spectrometry*, 32(3), 201-211.
- 855 [31] Zheng, W., Kollmeyer, J., Symolon, H., Momin, A., Munter, E., Wang, E., ... & Ramaraju, H.
 856 (2006). Ceramides and other bioactive sphingolipid backbones in health and disease: lipidomic
 857 analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy.
 858 *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1758(12), 1864-1884.
- 859 [32] t'Kindt, R., Jorge, L., Dumont, E., Couturon, P., David, F., Sandra, P., & Sandra, K. (2011).
 860 Profiling and characterizing skin ceramides using reversed-phase liquid chromatography–
 861 quadrupole time-of-flight mass spectrometry. *Analytical chemistry*, 84(1), 403-411.
- 862 [33] Castro, B. M., Prieto, M., & Silva, L. C. (2014). Ceramide: a simple sphingolipid with unique
 863 biophysical properties. *Progress in lipid research*, 54, 53-67.
- 864 [34] Xu, Y. (2018). Lysophospholipid signaling in the epithelial ovarian cancer tumor
 865 microenvironment. *Cancers*, 10(7), 227.
- 866 [35] Fujimoto, T., & Parton, R. G. (2011). Not just fat: the structure and function of the lipid
 867 droplet. *Cold Spring Harbor perspectives in biology*, 3(3), a004838.
- 868 [36] Welte, M. A. (2015). Expanding roles for lipid droplets. *Current biology*, 25(11), R470-R481.

- 869 [37] Welte, M. A., & Gould, A. P. (2017). Lipid droplet functions beyond energy storage.
870 *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1862(10), 1260-1272.
- 871 [38] Lydic, T. A., & Goo, Y. H. (2018). Lipidomics unveils the complexity of the lipidome in
872 metabolic diseases. *Clinical and translational medicine*, 7(1), 4.
- 873 [39] Liebisch, G., Ekroos, K., Hermansson, M., & Ejsing, C. S. (2017). Reporting of lipidomics
874 data should be standardized. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of*
875 *Lipids*, 1862(8), 747-751.
- 876 [40] Baker, P. R. S., "Current State of Quantitation in Lipidomics Analysis", Avanti Polar lipids
877 blog, 23.03.2019. [https://avantilipids.com/discussions/current-state-of-quantitation-in-lipidomics-](https://avantilipids.com/discussions/current-state-of-quantitation-in-lipidomics-analysis)
878 [analysis](https://avantilipids.com/discussions/current-state-of-quantitation-in-lipidomics-analysis)
- 879 [41] Ivanova, P. T., Milne, S. B., Byrne, M. O., Xiang, Y., & Brown, H. A. (2007).
880 Glycerophospholipid identification and quantitation by electrospray ionization mass spectrometry.
881 *Methods in enzymology*, 432, 21-57.
- 882 [42] Pi, J., Wu, X., & Feng, Y. (2016). Fragmentation patterns of five types of phospholipids by
883 ultra-high-performance liquid chromatography electrospray ionization quadrupole time-of-flight
884 tandem mass spectrometry. *Analytical Methods*, 8(6), 1319-1332.
- 885 [43] Sullards, M. C., Liu, Y., Chen, Y., & Merrill Jr, A. H. (2011). Analysis of mammalian
886 sphingolipids by liquid chromatography tandem mass spectrometry (LC-MS/MS) and tissue
887 imaging mass spectrometry (TIMS). *Biochimica et Biophysica Acta (BBA)-Molecular and Cell*
888 *Biology of Lipids*, 1811(11), 838-853.
- 889 [44] Khouri, Hania, "IM-QTOF Training", Agilent Technologies, 12.-18.01.2019 at the Department
890 of Technical chemistry (Johannes-Kepler University Linz)
- 891 [45] Ferchaud-Roucher, V., Croyal, M., Moyon, T., Zair, Y., Krempf, M., & Ouguerram, K. (2017).
892 Plasma lipidome analysis by liquid chromatography-high resolution mass spectrometry and ion

- 893 mobility of hypertriglyceridemic patients on extended-release nicotinic acid: a pilot study.
894 *Cardiovascular drugs and therapy*, 31(3), 269-279.
- 895 [46] Lísá, M., Cífková, E., Khalikova, M., Ovčáčíková, M., & Holčápek, M. (2017). Lipidomic
896 analysis of biological samples: comparison of liquid chromatography, supercritical fluid
897 chromatography and direct infusion mass spectrometry methods. *Journal of Chromatography A*,
898 1525, 96-108.
- 899 [47] Blaženović, I., Shen, T., Mehta, S. S., Kind, T., Ji, J., Piparo, M., Cacciola, F., Mondello, L., &
900 Fiehn, O. (2018). Increasing Compound Identification Rates in Untargeted Lipidomics Research
901 with Liquid Chromatography Drift Time–Ion Mobility Mass Spectrometry. *Analytical chemistry*,
902 90(18), 10758-10764.
- 903 [48] Hinz, C., Liggi, S., Mocciaro, G., Jung, S. M., Induruwa, I., Pereira, M. C., ... & Fjeldsted, J.
904 C. (2019). A comprehensive UHPLC ion mobility QTOF method for profiling and quantification of
905 eicosanoids, other oxylipins and fatty acids. *Analytical chemistry*.
- 906 [49] Wenk, M. R. (2010). Lipidomics: new tools and applications. *Cell*, 143(6), 888-895.
- 907 [50] Van Meulebroek, L., De Paepe, E., Vercruysse, V., Pomian, B., Bos, S., Lapauw, B., &
908 Vanhaecke, L. (2017). Holistic lipidomics of the human gut phenotype using validated ultra-high-
909 performance liquid chromatography coupled to hybrid orbitrap mass spectrometry. *Analytical*
910 *chemistry*, 89(22), 12502-12510.
- 911 [51] Rainville, P. D., Stumpf, C. L., Shockcor, J. P., Plumb, R. S., Nicholson, J. K. J. *Proteome Res.*
912 6 (2007) 552.
- 913 [52] Drotleff, B., Hallschmid, M., & Lämmerhofer, M. (2018). Quantification of steroid hormones
914 in plasma using a surrogate calibrant approach and UHPLC-ESI-QTOF-MS/MS with SWATH-
915 acquisition combined with untargeted profiling. *Analytica chimica acta*, 1022, 70-80.

- 916 [53] Gobo, L. A., de Carvalho, L. M., Temp, F., Viana, C., & Mello, C. F. (2018). A rapid method
917 for identification and quantification of prostaglandins in cerebral tissues by UHPLC-ESI-MS/MS
918 for the lipidomic in vivo studies. *Analytical biochemistry*, 545, 98-103.
- 919 [54] Capriotti, A. L., Cavaliere, C., & Piovesana, S. (2019). Liposome protein corona
920 characterization as a new approach in nanomedicine. *Analytical and bioanalytical chemistry*, 1-14.
- 921 [55] Hyötyläinen, T., & Orešič, M. (2015). Optimizing the lipidomics workflow for clinical
922 studies—practical considerations. *Analytical and bioanalytical chemistry*, 407(17), 4973-4993.
- 923 [56] Castro-Gómez, P., Montero, O., & Fontecha, J. (2017). In-depth lipidomic analysis of
924 molecular species of triacylglycerides, diacylglycerides, glycerophospholipids, and sphingolipids of
925 buttermilk by GC-MS/FID, HPLC-ELSD, and UPLC-QToF-MS. *International journal of*
926 *molecular sciences*, 18(3), 605.
- 927 [57] Lloyd, A. J., Beckmann, M., Wilson, T., Taillart, K., Allaway, D., & Draper, J. (2017). Ultra
928 high performance liquid chromatography–high resolution mass spectrometry plasma lipidomics can
929 distinguish between canine breeds despite uncontrolled environmental variability and non-
930 standardized diets. *Metabolomics*, 13(2), 15.
- 931 [58] Yuan, Z. X., Majchrzak-Hong, S., Keyes, G. S., Iadarola, M. J., Mannes, A. J., & Ramsden, C.
932 E. (2018). Lipidomic profiling of targeted oxylipins with ultra-performance liquid chromatography-
933 tandem mass spectrometry. *Analytical and bioanalytical chemistry*, 410(23), 6009-6029.
- 934 [59] Gao, X., Luo, J., Lü, L., Zhang, L., Zhang, S., & Cui, J. (2018). RNA-Seq and UHPLC-Q-
935 TOF/MS Based Lipidomics Study in *Lysiphlebia japonica*. *Scientific reports*, 8(1), 7802.
- 936 [60] Gupta, A., Dorlhiac, G. F., & Streets, A. M. (2019). Quantitative imaging of lipid droplets in
937 single cells. *Analyst*, 144(3), 753-765. nanoflow liquid chromatography-tandem mass spectrometry.
938 *Analytical chemistry*, 89(4), 2488-2496.
- 939 [61] Drotleff, B., Illison, J., Schlotterbeck, J., Lukowski, R., & Lämmerhofer, M. (2019).
940 Comprehensive Lipidomics of Mouse Plasma using Class-Specific Surrogate Calibrants and

- 941 SWATH Acquisition for Large-Scale Lipid Quantification in Untargeted Analysis. *Analytica*
 942 *Chimica Acta*.
- 943 [62] Zalloua, P., Kadar, H., Hariri, E., Farraj, L. A., Brial, F., Hedjazi, L., Lay, A. L., Colleum A.,
 944 Dubus, J., Touboul, D., Matsuda, F., Lathrop, M., Nicholson, J. K., Dumas, M. E. & Matsuda, F.
 945 (2019). Untargeted Mass Spectrometry Lipidomics identifies correlation between serum
 946 sphingomyelins and plasma cholesterol. *Lipids in health and disease*, 18(1), 38.
- 947 [63] Patterson, R. E., Kirpich, A. S., Koelmel, J. P., Kalavalapalli, S., Morse, A. M., Cusi, K.,
 948 Sunny, K., McIntyre, L. M., Garrett, T. J., & Yost, R. A. (2017). Improved experimental data
 949 processing for UHPLC–HRMS/MS lipidomics applied to nonalcoholic fatty liver disease.
 950 *Metabolomics*, 13(11), 142.
- 951 [64] Cheema, A., Mehta, K., Fatanmi, O., Wise, S., Hinzman, C., Wolff, J., & Singh, V. (2017). A
 952 Metabolomic and lipidomic serum signature from nonhuman primates administered with a
 953 promising radiation countermeasure, gamma-tocotrienol. *International journal of molecular*
 954 *sciences*, 19(1), 79.
- 955 [65] Liu, M. Y., Burgos, A., Ma, L., Zhang, Q., Tang, D., & Ruan, J. (2017). Lipidomics analysis
 956 unravels the effect of nitrogen fertilization on lipid metabolism in tea plant (*Camellia sinensis* L.).
 957 *BMC plant biology*, 17(1), 165.
- 958 [66] Lee, J. W., Mok, H. J., Lee, D. Y., Park, S. C., Kim, G. S., Lee, S. E., Lee, Y.S., Kim, K. P., &
 959 Kim, H. D. (2017). UPLC-QqQ/MS-based lipidomics approach to characterize lipid alterations in
 960 inflammatory macrophages. *Journal of proteome research*, 16(4), 1460-1469.
- 961 [67] Law, T. H., Volk, H. A., Pan, Y., Zanghi, B., & Want, E. J. (2018). Metabolic perturbations
 962 associated with the consumption of a ketogenic medium-chain TAG diet in dogs with idiopathic
 963 epilepsy. *British Journal of Nutrition*, 120(5), 484-490.

- 964 [68] He, Z., Wang, Y., Zhang, Y., Cheng, H., & Liu, X. (2018). Stereoselective bioaccumulation of
 965 chiral PCB 91 in earthworm and its metabolomic and lipidomic responses. *Environmental pollution*,
 966 238, 421-430.
- 967 [69] Chamberlain, C. A., Hatch, M., & Garrett, T. J. (2019). Metabolomic and lipidomic
 968 characterization of *Oxalobacter formigenes* strains HC1 and OxWR by UHPLC-HRMS. *Analytical*
 969 *and bioanalytical chemistry*, 1-12.
- 970 [70] Jeucken, A., & Brouwers, J. F. (2019). High-Throughput Screening of Lipidomic Adaptations
 971 in Cultured Cells. *Biomolecules*, 9(2), 42.
- 972 [71] Creydt, M., Vuralhan-Eckert, J., Fromm, J., & Fischer, M. (2019). Effects of elevated CO₂
 973 concentration on leaves and berries of black elder (*Sambucus nigra*) using UHPLC-ESI-QTOF-
 974 MS/MS and gas exchange measurements. *Journal of plant physiology*, 234, 71-79.
- 975 [72] Wang, X., Xu, Y., Song, X., Jia, Q., Zhang, X., Qian, Y., & Qiu, J. (2019). Analysis of
 976 glycerophospholipid metabolism after exposure to PCB153 in PC12 cells through targeted
 977 lipidomics by UHPLC-MS/MS. *Ecotoxicology and environmental safety*, 169, 120-127.
- 978 [73] Yang, J. S., Kim, J. Y., Lee, J. C., & Moon, M. H. (2019). Investigation of lipidomic
 979 perturbations in oxidatively stressed subcellular organelles and exosomes by asymmetrical flow
 980 field-flow fractionation and nanoflow ultrahigh performance liquid chromatography-tandem mass
 981 spectrometry. *Analytica chimica acta*, 1073, 79-89.
- 982 [74] Calderón, C., Rubarth, L., Cebo, M., Merfort, I., & Lämmerhofer, M. (2019). Lipid Atlas of
 983 Keratinocytes and Betulin Effects on its Lipidome Profiled by Comprehensive UHPLC- MS/MS
 984 With Data Independent Acquisition Using Targeted Data Processing. *Proteomics*, 1900113.
- 985 [75] Kappler, L., Hoene, M., Hu, C., von Toerne, C., Li, J., Bleher, D., Hoffmann, C., Böhm, A.,
 986 Kollipara, L., Zischka, H., Königsrainer, A., Häring, H. U., Peter, A., Xu, G., Sickmann, A.,
 987 Hauck, S. M., Weigert, C., & Lehmann, R. (2019). Linking bioenergetic function of mitochondria

- 988 to tissue-specific molecular fingerprints. *American Journal of Physiology-Endocrinology and*
 989 *Metabolism*.
- 990 [76] Fu, K., Wang, C., Gao, Y., Fan, S., Zhang, H., Sun, J., Jiang, Y., Liu, C., Guan, L., Liu, J.,
 991 Huang, M., & Bi, H. (2019). Metabolomics and lipidomics reveal the effect of hepatic Vps33b
 992 deficiency on bile acids and lipids metabolism. *Frontiers in Pharmacology*, 10, 276.
- 993 [77] Marqueño, A., Blanco, M., Maceda-Veiga, A., & Porte, C. (2019). Skeletal muscle lipidomics
 994 as a new tool to determine altered lipid homeostasis in fish exposed to urban and industrial
 995 wastewaters. *Environmental science & technology*.
- 996 [78] Hyötyläinen, T., & Orešič, M. (2014). Systems biology strategies to study lipidomes in health
 997 and disease. *Progress in lipid research*, 55, 43-60.
- 998 [79] Borowska-Strugiń, B., Druszczyńska, M., Lorkiewicz, W., Szewczyk, R., & Ządzińska, E.
 999 (2014). Mycolic acids as markers of osseous tuberculosis in the Neolithic skeleton from Kujawy
 1000 region (central Poland). *Anthropological review*, 77(2), 137-149.
- 1001 [80] Perrotti, F., Rosa, C., Cicalini, I., Sacchetta, P., Del Boccio, P., Genovesi, D., & Pieragostino,
 1002 D. (2016). Advances in lipidomics for cancer biomarkers discovery. *International journal of*
 1003 *molecular sciences*, 17(12), 1992.
- 1004 [81] Crick, P. J., & Guan, X. L. (2016). Lipid metabolism in mycobacteria—Insights using mass
 1005 spectrometry-based lipidomics. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology*
 1006 *of Lipids*, 1861(1), 60-67.
- 1007 [82] Daffé, M., & Draper, P. (1997). The envelope layers of mycobacteria with reference to their
 1008 pathogenicity. In *Advances in microbial physiology* (Vol. 39, pp. 131-203). Academic Press.
- 1009 [83] Zhang, Y., Liu, Y., Li, L., Wei, J., Xiong, S., & Zhao, Z. (2016). High resolution mass
 1010 spectrometry coupled with multivariate data analysis revealing plasma lipidomic alteration in
 1011 ovarian cancer in Asian women. *Talanta*, 150, 88-96.

- 1012 [84] Qadir, M. I., & Malik, S. A. (2008). Plasma lipid profile in gynecologic cancers. *European*
 1013 *journal of gynaecological oncology*, 29(2), 158.
- 1014 [85] Hu, C., van der Heijden, R., Wang, M., van der Greef, J., Hankemeier, T., & Xu, G. (2009).
 1015 Analytical strategies in lipidomics and applications in disease biomarker discovery. *Journal of*
 1016 *Chromatography B*, 877(26), 2836-2846.
- 1017 [86] Zhao, Z., Xiao, Y., Elson, P., Tan, H., Plummer, S. J., Berk, M., ... & Casey, G. (2007). Plasma
 1018 lysophosphatidylcholine levels: potential biomarkers for colorectal cancer. *Journal of clinical*
 1019 *oncology*, 25(19), 2696-2701.
- 1020 [87] Burgess, S., & Harshfield, E. (2016). Mendelian randomization to assess causal effects of
 1021 blood lipids on coronary heart disease: lessons from the past and applications to the future. *Current*
 1022 *opinion in endocrinology, diabetes, and obesity*, 23(2), 124.
- 1023 [88] Holmes, M. V., Ala-Korpela, M., & Smith, G. D. (2017). Mendelian randomization in
 1024 cardiometabolic disease: challenges in evaluating causality. *Nature Reviews Cardiology*, 14(10),
 1025 577.
- 1026 [89] Dong, S., Zhang, R., Liang, Y., Shi, J., Li, J., Shang, F., Mao, X., & Sun, J. (2017). Changes of
 1027 myocardial lipidomics profiling in a rat model of diabetic cardiomyopathy using UPLC/Q-TOF/MS
 1028 analysis. *Diabetology & metabolic syndrome*, 9(1), 56.
- 1029 [90] Wu, J., Wu, Q., Wang, D., Kong, J., Dai, W., Wang, X., & Yu, X. (2017). Common lipid
 1030 features of lethal ventricular tachyarrhythmias (LVTAs) induced by myocardial infarction and
 1031 myocardial ion channel diseases. *Scientific reports*, 7(1), 4220.
- 1032 [91] Chen, Y., Wen, S., Jiang, M., Zhu, Y., Ding, L., Shi, H., Dong, P., Yang, J., & Yang, Y.
 1033 (2017). Atherosclerotic dyslipidemia revealed by plasma lipidomics on ApoE^{-/-} mice fed a high-
 1034 fat diet. *Atherosclerosis*, 262, 78-86.
- 1035 [92] Yan, B., Chu, H., Yang, D., Sze, K. H., Lai, P. M., Yuan, S., Shuai, H., Wang, Y., Kao, R. Y.
 1036 T., Chan, J. F. W., & Yuen, K. Y. (2019). Characterization of the Lipidomic Profile of Human

- 1037 Coronavirus-Infected Cells: Implications for Lipid Metabolism Remodeling upon Coronavirus
 1038 Replication. *Viruses*, 11(1), 73.
- 1039 [93] Tietz-Bogert, P., Kim, M., Cheung, A., Tabibian, J., Heimbach, J., Rosen, C., Nandakumar,
 1040 M., Lazaridis, K. N., LaRusso, N. F., Sung, J. & O'Hara, S. (2018). Metabolomic Profiling of
 1041 Portal Blood and Bile Reveals Metabolic Signatures of Primary Sclerosing Cholangitis.
 1042 *International journal of molecular sciences*, 19(10), 3188.
- 1043 [94] Wu, J., Wu, Q., Dai, W., Kong, J., Lv, J., Yu, X., Wang, X., & Wang, D. (2018). Serum lipid
 1044 feature and potential biomarkers of lethal ventricular tachyarrhythmia (LVTA) induced by
 1045 myocardial ion channel diseases: a rat model study. *International journal of legal medicine*, 132(2),
 1046 439-448.
- 1047 [95] Chao, Y., Gao, S., Wang, X., Li, N., Zhao, H., Wen, X., Kou, Z., & Dong, X. (2018).
 1048 Untargeted lipidomics based on UPLC-QTOF-MS/MS and structural characterization reveals
 1049 dramatic compositional changes in serum and renal lipids in mice with glyoxylate-induced
 1050 nephrolithiasis. *Journal of Chromatography B*, 1095, 258-266.
- 1051 [96] Lee, J. H., Yang, J. S., Lee, S. H., & Moon, M. H. (2018). Analysis of lipoprotein-specific
 1052 lipids in patients with acute coronary syndrome by asymmetrical flow field-flow fractionation and
 1053 nanoflow liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, 1099,
 1054 56-63.
- 1055 [97] Kim, S. H., Yang, J. S., Lee, J. C., Lee, J. Y., Lee, J. Y., Kim, E., & Moon, M. H. (2018).
 1056 Lipidomic alterations in lipoproteins of patients with mild cognitive impairment and Alzheimer's
 1057 disease by asymmetrical flow field-flow fractionation and nanoflow ultrahigh performance liquid
 1058 chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1568, 91-100.
- 1059 [98] Cheema, A., Hinzman, C., Mehta, K., Hanlon, B., Garcia, M., Fatanmi, O., & Singh, V.
 1060 (2018). Plasma Derived Exosomal Biomarkers of Exposure to Ionizing Radiation in Nonhuman
 1061 Primates. *International journal of molecular sciences*, 19(11), 3427.

- 1062 [99] Shan, J., Qian, W., Shen, C., Lin, L., Xie, T., Peng, L., Xu, J., Yang, R., Ji, J., & Zhao, X.
 1063 (2018). High-resolution lipidomics reveals dysregulation of lipid metabolism in respiratory
 1064 syncytial virus pneumonia mice. *RSC Advances*, 8(51), 29368-29377.
- 1065 [100] Lee, H., Choi, J. M., Cho, J. Y., Kim, T. E., Lee, H. J., & Jung, B. H. (2018). Regulation of
 1066 endogenic metabolites by rosuvastatin in hyperlipidemia patients: An integration of metabolomics
 1067 and lipidomics. *Chemistry and physics of lipids*, 214, 69-83.
- 1068 [101] Wang, J., Zhang, L., Xiao, R., Li, Y., Liao, S., Zhang, Z., Yang, W., & Liang, B. (2019).
 1069 Plasma lipidomic signatures of spontaneous obese rhesus monkeys. *Lipids in health and disease*,
 1070 18(1), 8.
- 1071 [102] Lin, M., Wang, Z., Wang, D., Chen, X., & Zhang, J. (2019). A mathematical model-assisted
 1072 UHPLC-MS/MS method for global profiling and quantification of cholesteryl esters in
 1073 hyperlipidemic golden hamsters. *Analytical chemistry*.
- 1074 [103] Shan, J., Qian, W., Kang, A., Peng, L., Xie, T., Lin, L., Di, L., Xiao, P., & Zhou, W. (2019).
 1075 Lipid profile perturbations in the plasma and lungs of mice with LPS-induced acute lung injury
 1076 revealed by UHPLC-ESI-Q Exactive HF MS analysis. *Journal of pharmaceutical and biomedical*
 1077 *analysis*, 162, 242-248.
- 1078 [104] Li, J., Gao, Y., Guan, L., Zhang, H., Chen, P., Gong, X., Li, D., Liang, X., Huang, M., & Bi,
 1079 H. (2019). Lipid Profiling of Peri-implantation Endometrium in Patients with Premature
 1080 Progesterone Rise in the Late Follicular Phase. *The Journal of Clinical Endocrinology &*
 1081 *Metabolism*.
- 1082 [105] Gong, L. L., Yang, S., Zhang, W., Han, F. F., Lv, Y. L., Xuan, L. L., Liu, H., & Liu, L. H.
 1083 (2019). Discovery of Metabolite Profiles of Metabolic Syndrome Using Untargeted and Targeted
 1084 LC-MS based Lipidomics Approach. *Journal of pharmaceutical and biomedical analysis*, 112848.
- 1085 [106] Yang, R., Zhang, Y., Qian, W., Peng, L., Lin, L., Xu, J., Xie, T., Ji, J., & Shan, J. (2019).
 1086 Surfactant Lipidomics of Alveolar Lavage Fluid in Mice Based on Ultra-High-Performance Liquid

- 1087 Chromatography Coupled to Hybrid Quadrupole-Exactive Orbitrap Mass Spectrometry.
1088 Metabolites, 9(4), 80.
- 1089 [107] Li, Q., Liang, X., Zhao, L., Zhang, Z., Xue, X., Wang, K., & Wu, L. (2017). UPLC-Q-
1090 exactive orbitrap/MS-based lipidomics approach to characterize lipid extracts from bee pollen and
1091 their in vitro anti-inflammatory properties. *Journal of agricultural and food chemistry*, 65(32),
1092 6848-6860.
- 1093 [108] Zhang, H., Gao, Y., Sun, J., Fan, S., Yao, X., Ran, X., Zheng, C., Huang, M. & Bi, H. (2017).
1094 Optimization of lipid extraction and analytical protocols for UHPLC-ESI-HRMS-based lipidomic
1095 analysis of adherent mammalian cancer cells. *Analytical and bioanalytical chemistry*, 409(22),
1096 5349-5358.
- 1097 [109] Chao, H. C., Chen, G. Y., Hsu, L. C., Liao, H. W., Yang, S. Y., Wang, S. Y., Li, Y. L., Tang,
1098 S. C., Tseng, Y. J., & Kuo, C. H. (2017). Using precursor ion scan of 184 with liquid
1099 chromatography-electrospray ionization-tandem mass spectrometry for concentration normalization
1100 in cellular lipidomic studies. *Analytica chimica acta*, 971, 68-77.
- 1101 [110] Yang, J. S., Lee, J. C., Byeon, S. K., Rha, K. H., & Moon, M. H. (2017). Size dependent
1102 lipidomic analysis of urinary exosomes from patients with prostate cancer by flow field-flow
1103 fractionation and nanoflow liquid chromatography-tandem mass spectrometry. *Analytical*
1104 *chemistry*, 89(4), 2488-2496.
- 1105 [111] Li, Z., Guan, M., Lin, Y., Cui, X., Zhang, Y., Zhao, Z., & Zhu, J. (2017). Aberrant lipid
1106 metabolism in hepatocellular carcinoma revealed by liver lipidomics. *International journal of*
1107 *molecular sciences*, 18(12), 2550.
- 1108 [112] Lee, G. B., Lee, J. C., & Moon, M. H. (2019). Plasma lipid profile comparison of five
1109 different cancers by nanoflow ultrahigh performance liquid chromatography-tandem mass
1110 spectrometry. *Analytica Chimica Acta*.

- 1111 [113] Chen, Y., Ma, Z., Shen, X., Li, L., Zhong, J., Min, L. S., Xu, L., Li, H., Zhang, J., & Dai, L.
 1112 (2018). Serum lipidomics profiling to identify biomarkers for non-small cell lung cancer. *BioMed*
 1113 *research international*, 2018.
- 1114 [114] Jurowski, K., Kochan, K., Walczak, J., Barańska, M., Piekoszewski, W., & Buszewski, B.
 1115 (2017). Comprehensive review of trends and analytical strategies applied for biological samples
 1116 preparation and storage in modern medical lipidomics: State of the art. *TrAC Trends in Analytical*
 1117 *Chemistry*, 86, 276-289.
- 1118 [115] Patterson, R. E., Ducrocq, A. J., McDougall, D. J., Garrett, T. J., & Yost, R. A. (2015).
 1119 Comparison of blood plasma sample preparation methods for combined LC–MS lipidomics and
 1120 metabolomics. *Journal of Chromatography B*, 1002, 260-266.
- 1121 [116] Tumanov, S., & Kamphorst, J. J. (2017). Recent advances in expanding the coverage of the
 1122 lipidome. *Current opinion in biotechnology*, 43, 127-133.
- 1123 [117] López-Bascón, M. A., Calderón-Santiago, M., Sánchez-Ceinos, J., Fernández-Vega, A.,
 1124 Guzmán-Ruiz, R., López-Miranda, J., ... & Priego-Capote, F. (2018). Influence of sample
 1125 preparation on lipidomics analysis of polar lipids in adipose tissue. *Talanta*, 177, 86-93.
- 1126 [118] Rudralingam, Velauthan et al. “Ascites matters” *Ultrasound (Leeds, England)* vol. 25,2
 1127 (2016): 69-79.
- 1128 [119] Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and
 1129 purification of total lipides from animal tissues. *J biol Chem*, 226(1), 497-509.
- 1130 [120] Löfgren, L., Ståhlman, M., Forsberg, G. B., Saarinen, S., Nilsson, R., & Hansson, G. I.
 1131 (2012). The BUME method: a novel automated chloroform-free 96-well total lipid extraction
 1132 method for blood plasma. *Journal of lipid research*, 53(8), 1690-1700.
- 1133 [121] Lofgren, L., Stahlman, M., Forsberg, G. B., Saarinen, S., Nilsson, R., & Hansson, G. I.
 1134 (2012). The BUME method: a novel automated chloroform-free 96-well total lipid extraction
 1135 method for blood plasma. *Journal of lipid research*, jlr-D023036.

- 1136 [122] Drotleff, B., & Lämmerhofer, M. (2019). Guidelines for Selection of Internal Standard-based
1137 Normalization Strategies in Untargeted Lipidomic Profiling by LC-HR-MS/MS. *Analytical*
1138 *chemistry*.
- 1139 [123] Reis, A., Rudnitskaya et al. 2013 A comparison of five lipid extraction solvent systems for
1140 lipidomic studies of human LDL. *J Lipid Res*. 2013 Jul; 54(7): 1812–1824.
- 1141 [124] Cruz, M., Wang, M., Frisch- Daiello, J., & Han, X. (2016). Improved Butanol–Methanol
1142 (BUME) Method by Replacing Acetic Acid for Lipid Extraction of Biological Samples. *Lipids*,
1143 51(7), 887-896.
- 1144 [125] Teo, C. C., Chong, W. P. K., Tan, E., Basri, N. B., Low, Z. J., & Ho, Y. S. (2015). Advances
1145 in sample preparation and analytical techniques for lipidomics study of clinical samples. *TrAC*
1146 *Trends in Analytical Chemistry*, 66, 1-18.
- 1147 [126] Pulfer, M., & Murphy, R. C. (2003). Electrospray mass spectrometry of phospholipids. *Mass*
1148 *spectrometry reviews*, 22(5), 332-364.
- 1149 [127] Bang, G., Kim, Y. H., Yoon, J., Yu, Y. J., Chung, S., & Kim, J. A. (2017). On-Chip Lipid
1150 Extraction Using Superabsorbent Polymers for Mass Spectrometry. *Analytical chemistry*, 89(24),
1151 13365-13373.
- 1152 [128] Onorato, J. M., Shipkova, P., Minnich, A., Aubry, A. F., Easter, J., & Tymiak, A. (2014).
1153 Challenges in accurate quantitation of lysophosphatidic acids in human biofluids. *Journal of lipid*
1154 *research*, 55(8), 1784-1796. 1194/jlr.D050070
- 1155 [129] Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification.
1156 *Canadian journal of biochemistry and physiology*, 37(8), 911-917
- 1157 [130] Calderón, C., Sanwald, C., Schlotterbeck, J., Drotleff, B., & Lämmerhofer, M. (2019).
1158 Comparison of simple monophasic versus classical biphasic extraction protocols for comprehensive
1159 UHPLC-MS/MS lipidomic analysis of Hela cells. *Analytica chimica acta*, 1048, 66-74.

- 1160 [131] MacNair, J. E., Lewis, K. C., & Jorgenson, J. W. (1997). Ultrahigh-pressure reversed-phase
1161 liquid chromatography in packed capillary columns. *Analytical chemistry*, 69(6), 983-989.
- 1162 [132] Avela, H. F., Sirén, H., (2019), Analytical tools and current statistical methods used in mass
1163 spectrometry of glycerol-, glycerophospho- and sphingolipids. A review on liquid chromatographic
1164 measurements on-line coupled with various methods of mass spectrometry, *in-print*
- 1165 [133] Krautbauer, S., Büchler, C., & Liebisch, G. (2016). Relevance in the use of appropriate
1166 internal standards for accurate quantification using LC–MS/MS: tauro-conjugated bile acids as an
1167 example. *Analytical chemistry*, 88(22), 10957-10961.
- 1168 [134] Liebisch, G., The Role of LC–MS in Lipidomics. *LCGC Europe*, 2017, 30 240–242
- 1169 [135] Kartsova, L. A., Bessonova, E. A., & Somova, V. D. (2019). Hydrophilic Interaction
1170 Chromatography. *Journal of Analytical Chemistry*, 74(5), 415-424.
- 1171 [136] Erngren, I., Haglöf, J., Engskog, M. K., Nestor, M., Hedeland, M., Arvidsson, T., &
1172 Pettersson, C. (2019). Adduct formation in electrospray ionisation-mass spectrometry with
1173 hydrophilic interaction liquid chromatography is strongly affected by the inorganic ion
1174 concentration of the samples. *Journal of Chromatography A*, 1600, 174-182.
- 1175 [137] Lin, L., Yu, Q., Yan, X., Hang, W., Zheng, J., Xing, J., & Huang, B. (2010). Direct infusion
1176 mass spectrometry or liquid chromatography mass spectrometry for human metabonomics? A
1177 serum metabonomic study of kidney cancer. *Analyst*, 135(11), 2970-2978.
- 1178 [138] Fekete, S., Schappler, J., Veuthey, J. L., & Guilleme, D. (2014). Current and future trends in
1179 UHPLC. *TrAC Trends in Analytical Chemistry*, 63, 2-13.
- 1180 [139]
- 1181 [140] Danne-Rasche, N., Coman, C., & Ahrends, R. (2018). Nano-LC/ESI MS refines lipidomics
1182 by enhancing lipid coverage, measurement sensitivity, and linear dynamic range. *Analytical
1183 chemistry*, 90(13), 8093-8101.

- 1184 [141] Jeucken, A., Molenaar, M. R., van de Lest, C. H., Jansen, J. W., Helms, J. B., & Brouwers, J.
 1185 F. (2019). A comprehensive functional characterization of Escherichia coli lipid genes. *Cell reports*,
 1186 27(5), 1597-1606
- 1187 [142] Han, X., & Gross, R. W. (1994). Electrospray ionization mass spectroscopic analysis of
 1188 human erythrocyte plasma membrane phospholipids. *Proceedings of the National Academy of*
 1189 *Sciences*, 91(22), 10635-10639.
- 1190 [143] Holcapek, M., Liebisch, G., & Ekroos, K. (2018). Lipidomic Analysis.
- 1191 [144] Yang, K., & Han, X. (2016). Lipidomics: techniques, applications, and outcomes related to
 1192 biomedical sciences. *Trends in biochemical sciences*, 41(11), 954-969.
- 1193 [145] Kohlwein, S. D. (2017). Opinion articles on lipidomics-A critical assessment of the state-of-
 1194 the-art. *Biochimica et biophysica acta. Molecular and cell biology of lipids*, 1862(8), 729.
- 1195 [146] Sethi, S., & Brietzke, E. (2017). Recent advances in lipidomics: Analytical and clinical
 1196 perspectives. *Prostaglandins & other lipid mediators*, 128, 8-16.
- 1197 [147] Calderón, C., Sanwald, C., Schlotterbeck, J., Drotleff, B., & Lämmerhofer, M. (2019).
 1198 Comparison of simple monophasic versus classical biphasic extraction protocols for comprehensive
 1199 UHPLC-MS/MS lipidomic analysis of Hela cells. *Analytica chimica acta*, 1048, 66-74.
- 1200 [148] Zhang, J., Rector, J., Lin, J. Q., Young, J. H., Sans, M., Katta, N., Giese, N., Yu, W., Nagi,
 1201 C., Suliburk, J., Liu, J., Bensussan, A.m DeHoog, R. J., Garza, K. Y., Ludolph, B., Sorace, A. G.,
 1202 Syed, A., Zahedivash, A., Milner, T. E., & Eberlin, L. S. (2017). Nondestructive tissue analysis for
 1203 ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. *Science*
 1204 *translational medicine*, 9(406), ean3968.
- 1205 [149] Phelps, D. L., Balog, J., Gildea, L. F., Bodai, Z., Savage, A., El-Bahrawy, M. A., Speller, A.
 1206 V. M., Rosini, F., Kudo, H., McKenzie, J. S., Brown, R., Takáts, Z., & Brown, R. (2018). The
 1207 surgical intelligent knife distinguishes normal, borderline and malignant gynaecological tissues

- 1208 using rapid evaporative ionisation mass spectrometry (REIMS). *British journal of cancer*, 118(10),
1209 1349.
- 1210 [150] Avela, H. F. (2019). Recent Developments and Challenges in Chromatographic Lipidomics.
1211 Helsinki University, E-thesis
- 1212 [151] Lorenzen, W., Bozhüyük, K. A., Cortina, N. S., & Bode, H. B. (2014). A comprehensive
1213 insight into the lipid composition of *Myxococcus xanthus* by UPLC-ESI-MS. *Journal of lipid*
1214 *research*, 55(12), 2620-2633.
- 1215 [152] Cajka, T., & Fiehn, O. (2014). Comprehensive analysis of lipids in biological systems by
1216 liquid chromatography-mass spectrometry. *TrAC Trends in Analytical Chemistry*, 61, 192-206.
- 1217 [153] Merrill Jr, A. H., Sullards, M. C., Allegood, J. C., Kelly, S., & Wang, E. (2005).
1218 Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids
1219 by liquid chromatography tandem mass spectrometry. *Methods*, 36(2), 207-224.
- 1220 [154] Lemke, A., Castillo-Sánchez, J. C., Prodinger, F., Ceranic, A., Hennerbichler-Lugscheider, S.,
1221 Pérez-Gil, J., Redl, H. & Wolbank, S. (2017). Human amniotic membrane as newly identified
1222 source of amniotic fluid pulmonary surfactant. *Scientific reports*, 7(1), 6406.
- 1223 [155] Xuan, Q., Hu, C., Yu, D., Wang, L., Zhou, Y., Zhao, X., Li, Q, Hou, X. & Xu, G. (2018).
1224 Development of a High Coverage Pseudotargeted Lipidomics Method Based on Ultra-High
1225 Performance Liquid Chromatography–Mass Spectrometry. *Analytical chemistry*, 90(12), 7608-
1226 7616.
- 1227 [156] De Paepe, E., Van Meulebroek, L., Rombouts, C., Huysman, S., Verplanken, K., Lapauw, B.,
1228 Wauters, J., Hemeryck, L. Y., Vanhaecke, L., & Vanhaecke, L. (2018). A validated multi-matrix
1229 platform for metabolomic fingerprinting of human urine, feces and plasma using ultra-high
1230 performance liquid-chromatography coupled to hybrid orbitrap high-resolution mass spectrometry.
1231 *Analytica chimica acta*, 1033, 108-118.

- 1232 [157] Ulmer, C. Z., Jones, C. M., Yost, R. A., Garrett, T. J., & Bowden, J. A. (2018). Optimization
1233 of Folch, Bligh-Dyer, and Matyash sample-to-extraction solvent ratios for human plasma-based
1234 lipidomics studies. *Analytica chimica acta*, 1037, 351-357.
- 1235 [158] Byeon, S. K., Kim, J. Y., Lee, J. Y., Chung, B. C., Seo, H. S., & Moon, M. H. (2015). Top-
1236 down and bottom-up lipidomic analysis of rabbit lipoproteins under different metabolic conditions
1237 using flow field-flow fractionation, nanoflow liquid chromatography and mass spectrometry.
1238 *Journal of Chromatography A*, 1405, 140-148.
- 1239 [159] Chang, W. Q., Zhou, J. L., Li, Y., Shi, Z. Q., Wang, L., Yang, J., Li, P., Liu, L. F., & Xin, G.
1240 Z. (2017). An in vitro approach for lipolysis measurement using high-resolution mass spectrometry
1241 and partial least squares based analysis. *Analytica chimica acta*, 950, 138-146.
- 1242 [160] Triebel, A., Trötz Müller, M., Hartler, J., Stojakovic, T., & Köfeler, H. C. (2017). Lipidomics
1243 by ultrahigh performance liquid chromatography-high resolution mass spectrometry and its
1244 application to complex biological samples. *Journal of Chromatography B*, 1053, 72-80.
- 1245 [161] Witting, M., Ruttkies, C., Neumann, S., & Schmitt-Kopplin, P. (2017). LipidFrag: Improving
1246 reliability of in silico fragmentation of lipids and application to the *Caenorhabditis elegans*
1247 lipidome. *PloS one*, 12(3), e0172311.
- 1248 [162] Ejsing, C. S. (2007). Molecular characterization of the lipidome by mass spectrometry
1249 (Doctoral dissertation, Verlag nicht ermittelbar).
- 1250 [163] Blaas, N., & Humpf, H. U. (2013). Structural profiling and quantitation of glycosyl inositol
1251 phosphoceramides in plants with Fourier transform mass spectrometry. *Journal of agricultural and*
1252 *food chemistry*, 61(18), 4257-4269.
- 1253 [164] S Elkhayat, E., A Mohamed, G., & RM Ibrahim, S. (2012). Activity and structure elucidation
1254 of ceramides. *Current Bioactive Compounds*, 8(4), 370-409.

- 1255 [165] Scherer M., Schmitz G., Liebisch G. 2010. Simultaneous quantification of cardiolipin,
1256 bis(monoacylglycero)phosphate and their precursors by hydrophilic interaction LC-MS/MS
1257 including correction of isotopic overlap. *Anal. Chem.* 82: 8794–8799.
- 1258 [166] Berry, Karin A. Zemski, and Robert C. Murphy. "Electrospray ionization tandem mass
1259 spectrometry of glycerophosphoethanolamine plasmalogen phospholipids." *Journal of the American*
1260 *Society for Mass Spectrometry* 15.10 (2004): 1499-1508.
- 1261 [167] Hsu, F. F., Turk, J., Rhoades, E. R., Russell, D. G., Shi, Y., & Groisman, E. A. (2005).
1262 Structural characterization of cardiolipin by tandem quadrupole and multiple-stage quadrupole ion-
1263 trap mass spectrometry with electrospray ionization. *Journal of the American Society for Mass*
1264 *Spectrometry*, 16(4), 491-504.
- 1265 [168] Smith, P. B., Snyder, A. P., & Harden, C. S. (1995). Characterization of bacterial
1266 phospholipids by electrospray ionization tandem mass spectrometry. *Analytical chemistry*, 67(11),
1267 1824-1830.
- 1268 [169] Ring, M. W., Schwär, G., & Bode, H. B. (2009). Biosynthesis of 2- hydroxy and iso- even
1269 fatty acids is connected to sphingolipid formation in myxobacteria. *ChemBioChem*, 10(12), 2003-
1270 2010.
- 1271 [170] Curtis, P. D., Geyer, R., White, D. C., & Shimkets, L. J. (2006). Novel lipids in *Myxococcus*
1272 *xanthus* and their role in chemotaxis. *Environmental microbiology*, 8(11), 1935-1949.
- 1273 [171] Kleinig, H. (1972). Membranes from *Myxococcus fulvus* (myxobacterales) containing
1274 carotenoid glucosides: I. Isolation and composition. *Biochimica et Biophysica Acta (BBA)-*
1275 *Biomembranes*, 274(2), 489-498.
- 1276 [172] Orndorff, P. E., & Dworkin, M. (1980). Separation and properties of the cytoplasmic and
1277 outer membranes of vegetative cells of *Myxococcus xanthus*. *Journal of bacteriology*, 141(2), 914-
1278 927.

- 1279 [173] Murphy, R. C., James, P. F., McAnoy, A. M., Krank, J., Duchoslav, E., & Barkley, R. M.
1280 (2007). Detection of the abundance of diacylglycerol and triacylglycerol molecular species in cells
1281 using neutral loss mass spectrometry. *Analytical biochemistry*, 366(1), 59-70.
- 1282 [174] Rampler, E., Coman, C., Hermann, G., Sickmann, A., Ahrends, R., & Koellensperger, G.
1283 (2017). LILY-lipidome isotope labeling of yeast: in vivo synthesis of ¹³C labeled reference lipids
1284 for quantification by mass spectrometry. *Analyst*, 142(11), 1891-1899.
- 1285 [175] Wenk, M. R. (2005). The emerging field of lipidomics. *Nature reviews Drug discovery*, 4(7),
1286 594.
- 1287 [176] Blanksby, S. J., & Mitchell, T. W. (2010). Advances in mass spectrometry for lipidomics.
1288 *Annual Review of Analytical Chemistry*, 3, 433-465.
- 1289 [177] Liebisch, G., Vizcaíno, J. A., Köfeler, H., Trötz Müller, M., Griffiths, W. J., Schmitz, G., ... &
1290 Wakelam, M. J. (2013). Shorthand notation for lipid structures derived from mass spectrometry.
1291 *Journal of lipid research*, 54(6), 1523-1530.
- 1292 [178] Pham, T. H., Zaeem, M., Fillier, T. A., Nadeem, M., Vidal, N. P., Manful, C., Cheema, S.,
1293 Cheema, M., & Thomas, R. H. (2019). Targeting Modified Lipids during Routine Lipidomics
1294 Analysis using HILIC and C30 Reverse Phase Liquid Chromatography coupled to Mass
1295 Spectrometry. *Scientific reports*, 9(1), 5048.
- 1296 [179] Gorrochategui, E., Jaumot, J., Lacorte, S., & Tauler, R. (2016). Data analysis strategies for
1297 targeted and untargeted LC-MS metabolomic studies: Overview and workflow. *TrAC Trends in*
1298 *Analytical Chemistry*, 82, 425-442.
- 1299 [180] Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and
1300 powerful approach to multiple testing. *Journal of the Royal statistical society: series B*
1301 *(Methodological)*, 57(1), 289-300.

- 1302 [181] Fu, K., Wang, C., Gao, Y., Fan, S., Zhang, H., Sun, J., Jiang, Y., Liu, J., Huang, M., & Bi, H.
 1303 (2019). Metabolomics and lipidomics reveal the effect of hepatic Vps33b deficiency on bile acids
 1304 and lipids metabolism. *Frontiers in Pharmacology*, 10, 276.
- 1305 [182] Storey, J. D. (2003). The positive false discovery rate: a Bayesian interpretation and the q-
 1306 value. *The Annals of Statistics*, 31(6), 2013-2035.
- 1307 [183] Nachar, N. (2008). The Mann-Whitney U: A test for assessing whether two independent
 1308 samples come from the same distribution. *Tutorials in quantitative Methods for Psychology*, 4(1),
 1309 13-20.
- 1310 [184] Koelmel, J. P., Kroeger, N. M., Ulmer, C. Z., Bowden, J. A., Patterson, R. E., Cochran, J. A.,
 1311 Beecher, C. W. W., Garrett, T. J. & Yost, R. A. (2017). LipidMatch: an automated workflow for
 1312 rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data.
 1313 *BMC bioinformatics*, 18(1), 331.
- 1314 [185] Kind, T., Liu, K. H., Lee, D. Y., DeFelice, B., Meissen, J. K., & Fiehn, O. (2013). LipidBlast
 1315 in silico tandem mass spectrometry database for lipid identification. *Nature methods*, 10(8), 755.
- 1316 [186] Lin, L., Yu, Q., Yan, X., Hang, W., Zheng, J., Xing, J., & Huang, B. (2010). Direct infusion
 1317 mass spectrometry or liquid chromatography mass spectrometry for human metabonomics? A
 1318 serum metabonomic study of kidney cancer. *Analyst*, 135(11), 2970-2978.
- 1319 [187] Smith, R., Mathis, A. D., Ventura, D., & Prince, J. T. (2014). Proteomics, lipidomics,
 1320 metabolomics: a mass spectrometry tutorial from a computer scientist's point of view. *BMC*
 1321 *bioinformatics*, 15(7), S9.
- 1322 [188] Griffiths, W. J., & Wang, Y. (2017). Sterolomics: State of the art, developments, limitations
 1323 and challenges. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*,
 1324 1862(8), 771-773.
- 1325 [189] Beccaria, M., Inferrera, V., Rigano, F., Gorynski, K., Purcaro, G., Pawliszyn, J., Dugo, P., &
 1326 Mondello, L. (2017). Highly informative multiclass profiling of lipids by ultra-high performance

liquid chromatography–Low resolution (quadrupole) mass spectrometry by using electrospray ionization and atmospheric pressure chemical ionization interfaces. *Journal of Chromatography A*, 1509, 69-82.

[190] Kim, H. S., Waqued, S. C., Nodurft, D. T., Devarenne, T. P., Yakovlev, V. V., & Han, A. (2017). Raman spectroscopy compatible PDMS droplet microfluidic culture and analysis platform towards on-chip lipidomics. *Analyst*, 142(7), 1054-1060.

List of Figures

Figure 1. Fatty acid elongation and desaturation processes by a *de novo* synthesis and b dietary uptake. The n-families are determined by the position of the double bond. Ref. [16]

Figure 2. FAs, [12] GL and GP [16] molecular characteristics. Acyl carnitine (AcCA) and cardiolipin (CL) structures are depicted to remind of more complex and less understood lipid structures.

Figure 3. Typical sphingolipid (SP) structures. R1 is an amine that usually has a fatty acid linked to it with a nitrogen bond. R2 on the other hand is either a free hydroxyl group as in ceramides but occupied with a characteristic phosphorylated molecule in sphingomyelins (SMs). Structures derived from ref. [17].

Figure 4. Fragmentation of three typical GPs as reported by Ivanova et al. [41] Naturally, the exact mass should be used for the proper identification of a “lipid class-selective fragment” [39]. Also, the de-/protonated state of the ionized lipid species and neutral losses should be considered.

Figure 5. Fragmentation patterns of SPs with denoted sites of cleavage (for S and Cer) [153]. Note that SA fragments are nearly identical with sphingosine species, except they have two more hydrogen and no DB in the backbone ($M_{SA}=M_S+2$). Ref. [43]

Figure 6. Combined base peak chromatogram and contour plot of HeLa lipids during exponential cell growth. Cells were lysed in chloroform / methanol and the resulting extract was injected directly on the high-performance liquid chromatography (HPLC) column after removal of precipitated proteins and macro-molecules by centrifugation (REF [70])

1353 *Figure 7.* Structures of (a) nonpolar and (b) polar lipid subclass in separation techniques. Reprinted
 1354 with permission from ref [116], DOI: 0958-1669/ 2016. S Tumanov and JJ Kamphorst. Published
 1355 by Elsevier Ltd, an open access article under the CC BY license.

1356 *Figure 8.* Product ion scans of negative mode HILIC-separated lipids from a standard mix
 1357 (molecular ion is marked with an arrow). Reprinted from ref [178] © 2019, Springer Nature
 1358 MMPE: monomethyl-phosphatidylethanolamine, DMPE: dimethyl-phosphatidylethanolamine

1359 *Figure 9.* Creation, validation, and application of *in-silico* generated MS/MS spectra in LipidBlast.
 1360 (a) New lipid compound structures were generated using *in-silico* methods. Lipid core structure
 1361 scaffolds were connected *via* a linker to fatty acyls with different chain lengths and different
 1362 degrees of unsaturation. Asterisks denote connection points. (b) Reference tandem spectra (top)
 1363 were used to simulate mass spectral fragmentations and ion abundances of the *in-silico* spectra
 1364 (bottom). The compound shown is PC(16:0/ 16:1) at precursor m/z 732.55 $[M + H]^+$. (c) For lipid
 1365 identification, MS/MS spectra obtained from LC-MS/MS or direct-infusion experiments were
 1366 submitted to LipidBlast. Ref. [185]

1367 *Figure 10.* LipidBlast was mostly developed with ion trap tandem mass spectra but can be used
 1368 with data from other platforms such as QTOF mass spectrometers. a) The Cardiolipin example
 1369 shows that even in the in the case of the non-matching but abundant precursor ion at m/z 1239.8355
 1370 $[M-H]^-$, the correct result is obtained with LipidBlast. b) The standard reference compound with
 1371 precursor $m/z=793.4841$ $[M-H]^-$ is correctly identified as phosphatidylinositol PI(17:0/14:1) as first
 1372 hit in a library search with LipidBlast. Ref. [185]

1373

1374

1375 **List of Tables**

1376 *Table 1.* Research on homeostatic profiling (10 articles)

1377 *Table 2.* Metabolomics research topics. (16 articles)

- 1378 *Table 3.* UHPLC-research on lipidomic pathway-based diseases and potential biomarkers
 1379 (19 articles)
- 1380 *Table 4.* Lipidomic UHPLC-research focusing on cancer
- 1381 *Table 5.* Extraction and sample purification methods used in lipidomics.
- 1382 *Table 6.* Molecular species formed during electrospray ionization of lipids. Ref. [152]
- 1383 *Table 7.* Classical biological material types. Extension to Jurovski et al.'s Table (Ref. [114]).
- 1384 *Table 8.* Alternative biological materials. Extension to Supplementary Table 1 by Jurovski et al.
 1385 [114] Hex is an abbreviation for hexosyl, Hex2 for dihexosyl
- 1386 *Table 9.* Cell culture matrices analyzed with UHPLC-MS techniques.
 1387 Hex is an abbreviation for hexosyl, Hex2 for dihexosyl
- 1388 *Table 10.* Common lipids according to research topic and identification approach. When
 1389 "Identified lipids" are indicated as a sum without any explanation, it means that the first number is
 1390 the amount of lipids found in positive and the second in negative mode (positive+negative).
- 1391 *Table 11.* Extension of lipid classes analyzed in different research articles (2017-2019)

Table 1. Research on homeostatic profiling (10 articles)

Year	Citation	Theme	Subject
2017	[50]	Homeostatic profiling	Human gut phenotype profiling
2017	[56]	Homeostatic profiling	Buttermilk profiling
2017	[57]	Homeostatic profiling	Distinguishing between canine breeds
2018	[58]	Homeostatic profiling	Oxylipin analysis in human patients
2018	[15]	Homeostatic profiling	SM and Cer in multiple tissues/cell cultures of humans, dogs, mice and rats
2018	[59]	Homeostatic profiling	Larvae profiling (<i>Lysphlebia japonica</i>)
2018	[52]	Homeostatic profiling	Steroid hormone quantification in human plasma
2018	[53]	Homeostatic profiling	<i>In vivo</i> prostaglandin identification and quantitation in human brain tissue
2019	[60]	Homeostatic profiling	Coherent Raman scattering (CRS), non-destructive lipid/metabolite profiling
2019	[61]	Homeostatic profiling	Mouse plasma analysis with matrix-matched calibrants and SWATH* MS/MS

*SWATH: sequential window acquisition of all theoretical fragment ion mass spectra

Table 2. Metabolomics research topics. (16 articles)

Year	Citation	Theme	Subject
2017	[63]	Applications / Metabolomics	Nonalcoholic fatty liver disease profiling, improved data-analysis
2017	[64]	Metabolomics	Radiation countermeasure mechanism, GT3 inhibitor test
2017	[65]	Metabolomics	Fertilization of plants
2017	[66]	Metabolomics	Inflammatory macrophage characterization
2018	[67]	Metabolomics	Ketogenic diet, consequent metabolic perturbations, odd carbon lipids
2018	[68]	Metabolomics	Bioaccumulation & metabolomic response to chiral PCB 91
2019	[62]	Metabolomics	Correlation between serum SMs and plasma cholesterol
2019	[69]	Metabolomics	Oxalobacter profiling for oxalate-based disease research
2019	[70]	Metabolomics	High-throughput 96-well cell culture assay
2019	[71]	Metabolomics	Elevated CO ₂ concentration, leaves & berries of the black elder plant
2019	[72]	Metabolomics	Lipido-metabolic changes upon PCB153/PC12 exposure
2019	[73]	Metabolomics	Lipidomic perturbations of HEK 293 cells and exosomes under oxidative stress
2019	[74]	Metabolomics	Keratinocyte lipidomics affected by botulin
2019	[75]	Metabolomics	Analysis on the cellular functions of liver and skeletal muscle mitochondria
2019	[76]	Metabolomics	Targeted bile acid analysis for validation of liver cholestasis-associated protein
2019	[87]	Metabolomics	Skeletal muscle analysis of urban industrial waste water-exposed fish*

**B. meridionalis* and *S. laietanus*

Table 3. UHPLC-research on lipidomic pathway-based diseases and potential biomarkers

(19 articles)

Year	Citation	Theme	Subject
2017	[89]	Diseases and Biomarkers	Diabetic cardiomyopathy
2017	[90]	Diseases and Biomarkers	Lethal ventricular tachyarrhythmia induced by myocardial ion channel diseases & infarction
2017	[91]	Diseases and Biomarkers	Atherosclerotic dyslipidemia via a high-fat diet on mice
2018	[92]	Diseases and Biomarkers	<i>In vitro</i> Coronavirus-infection of cell cultures
2018	[93]	Diseases and Biomarkers	Primary sclerosing cholangitis
2018	[94]	Diseases and Biomarkers	Lethal ventricular tachyarrhythmia induced by myocardial ion channel diseases
2018	[95]	Diseases and Biomarkers	Glyoxylate-induced nephrolithiasis
2018	[96]	Diseases and Biomarkers	Acute coronary syndrome
2018	[97]	Diseases and Biomarkers	Alzheimer's disease and amnesic mild cognitive impairment
2018	[98]	Diseases and Biomarkers	Plasma derived exosomal biomarkers, radiation exposure
2018	[99]	Diseases and Biomarkers	Dysregulation in respiratory syncytial virus pneumonia (mouse)
2018	[100]	Diseases and Biomarkers	Regulation of rosuvastatin in lipidemia patients
2019	[101]	Diseases and Biomarkers	Obesity biomarkers (rhesus monkey)
2019	[102]	Diseases and Biomarkers	VLDL, LDL and HDL CEs during lipidemia (golden hamster), unsaturation correlation with logarithmic mathematical model
2019	[103]	Diseases and Biomarkers	GL-induced acute lung injury (lipopolysaccharides, mouse)
2019	[9]	Diseases and Biomarkers	Blood platelets in coronary artery disease
2019	[104]	Diseases and Biomarkers	Premature progesterone rise affecting women's endometrium
2019	[105]	Diseases and Biomarkers	Plasma-analysis of Metabolic syndrome
2019	[106]	Diseases and Biomarkers	Alveolar lavage fluid analysis, acute lung injury

Table 4. Lipidomic UHPLC-research focusing on cancer

Year	Citation	Theme	Subject
2017	[46]	Applications / Cancer research	Kidney cancer, human patients; Comparison of LC/MS;SCF/MS;DIMS
2017	[107]	Applications / Cancer research	Bee pollen anti-inflammatory properties on cancer cells
2017	[108]	Applications / Cancer research	Lipid extraction comparison with pancreatic cancer cell line
2017	[109]	Applications / Cancer research	Cancer cell lines, Quantitative analysis, PC/SM PIS-184 optimization
2017	[110]	Cancer research	Urinary exosomes in prostate cancer patients
2017	[111]	Cancer research	Hepatocellular carcinoma in cancer patients
2018	[112]	Cancer research	Review comparing plasmalipid profiles of liver, lung, gastric, colorectal and thyroid cancer, nanoflow UHPLC
2018	[113]	Cancer research	Non-small cell lung cancer serum biomarker identification
2019	[8]	Cancer research	Colorectal cancer, Validation of colon cell/tissue analysis

Table 5. Extraction and sample purification methods used in lipidomics.

Class	Technique	Citation
Polarity-based	Single solvent extractions	[51], [128], [130]
Polarity-based	Liquid-liquid extractions	[51], [128], [130]
Polarity-based	Single solvent mixtures	[51], [128]
Polarity-based	Bligh and Dyer	[129]
Polarity-based	BUME	[120]
Polarity-based	Folch	[119]
Polarity-based	MTBE	[55]
Polarity-based	QuEChERS	[127]
Polarity-based	Supercritical fluid extraction (SFE)	[114], [125]
Polarity-based	Solid phase extraction (SPE)	[58], [125]
Polarity-based	Superabsorbent polymer extraction (SPE)	[127]
Energy-based	Microwave-assisted extraction (MAE)	[114], [125]
Energy-based	Ultrasonic-assisted extraction (UAE)	[114], [125]
Energy-based	Pressurized liquid extraction	[114], [125]

Table 6. Molecular species formed during electrospray ionization of lipids. Ref. [152]

Lipid class	Positive mode	Negative mode
LPC, PC	$[M + H]^+$, $[M + Na]^+$	$[M-H]^-$, $[M + HCOO]^-$, $[M + CH_3COO]^-$
LPE, PE	$[M + H]^+$, $[M + Na]^+$	$[M-H]^-$
PG	$[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$	$[M-H]^-$
PI	$[M + H]^+$, $[M + NH_4]^+$	$[M-H]^-$
PS	$[M + H]^+$	$[M-H]^-$
PA		$[M-H]^-$
CE	$[M + NH_4]^+$	
SM	$[M + H]^+$	$[M + HCOO]^-$
Cholesterol	$[M-H_2O+H]^+$	
Cer, GluCer, LacCer	$[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$	$[M-H]^-$, $[M + HCOO]^-$
MG, DG, TG	$[M + NH_4]^+$, $[M + Na]^+$	
MGDG, DGDG, SQDG	$[M + NH_4]^+$, $[M + Na]^+$	$[M-H]^-$
Fatty acids		$[M-H]^-$
CL	$[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$	$[M-H]^-$
Cer	$[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$	$[M-H]^-$

Table 7. Classical biological material types. Extension to Jurowski et al.'s Table (Ref. [114]).

Biological samples		Lipid classes	Reference(s)
classical biological materials (conventional)	Urine	AcCa, MG, DG, TG, PC, PE, LPC, Cer, SM, CEs	[5], [110]
	faecal matter	FA, GPs, STs, SPs, PRs, PKs	[50], [156]
	blood derivatives:		
	Plasma	FA, MG, DG, TG, PA, PC(+PLs), PE(+PLs), PI, PS, LPA, LPC, LPE, Cer(+Hex/Hex2/sulfoHex), SM, S, SA, cholesterol, CEs, SLs, STs	[5], [46], [52], [57], [58], [61], [62], [64], [67], [189], [91], [96], [99], [100], [102], [103], [105], [155], [157]*, [158]
	Serum	MG, DG, TG, PA, PC(+PLs), PE(+PLs), PG, PI, PS, LPA, LPC, LPE, Cer(+HexCer/Hex2Cer), SM, S, CEs, STs, PRs	[5], [15], [62], [76], [90], [94], [95], [113]
	Erythrocytes	PC(+PLs), PE(+PLs), LPC, LPE, Hex2Cer, SM	[46]
	blood platelets	AcCa, FA, DG, TG, PA, PC, PE, PG, PI, PS, LPA, LPC, LPE, Cer, SM	[9], [48]**

*NIST SRM 1950 plasma, ** Salmonella enterica serovar Typhimurium-infected murine bonemarrow-derived macrophages and thrombin activated blood platelets (human)

Hex: for hexosyl, **Hex2:** dihexosyl, **S:** sphingosine, **SA:** sphinganine

Table 8. Alternative bioical materials. Extension to Supplementary Table 1 by Jurovski et al. [114] Hex is an abbreviation for hexosyl, Hex2 for dihexosyl

Biological samples		Lipid classes	Reference(s)
alternative biological materials (unconventional)	Aqueous humor		[5], [127]
	Bile		[93]
	Bronchoalveolar lavage fluid	FA, GLs, GPs, OxGPs, LPLs, SLs	[106]
	Cerebrospinal fluid (CSF)		[5]
	Colon derived suspension		[8]
	Milk	DG, TG, PC, SM	[47]
	Tear drops		[5]
	Cells and cell lines:		
	x exosomes	FA, GLs, OxGPs, LPLs, SPs, HexCer, CEs, STs	[5], [73], [98], [110]
	x liposomes	FA	[159]
	x alveolar cells	PC, PE(+PLs), PS, LPLs, SM, Cer, HexCer	[160]
	x lipid droplets		[60]
	x ceratinocytes	FA, GLs, GPs, OxGPs, LPLs, SPs, HexCer, CEs	[74]
	Tissues:		
	x adipose tissue	Cer, SM	[15]
	x lung tissue	AcCa, FA, TG, PC(+PLS), PE(+PLs), PG, PI, PS, LPC, LPE, LPI, LPS, Cer, SLs	[99], [103]
	x renal tissue	FA, GLs, GPs, SLs, STs, PRs	[95]
	x myocardial tissue	FA, CL, DG, TG, PA, PC, PE, PG, PI, PS LPC, LPE, Cer, cholesterol	[89], [90]
	x brain tissue	Cer, SM, thromboxane, prostaglandins	[15], [53], [155]

	x endometrium	GLs, GPs, LPLs, SPs	[104]
	x liver tissue	DG, TG, PA, PC(+PLs), PE(+PLs), PG, PI, PS, LPA, LPC, LPE, LPG, LPI, LPS, Cer(+HexCer), SM, STs	[15], [63], [76], [111], [127], [155], [160]
	x colonic tissue		[8]
	x tumor tissue	PC(+PLs), PE(+PLs), LPC, LPE, Hex2Cer, SM	[46]

Table 9. Cell culture matrices analyzed with UHPLC-MS techniques.

Hex is an abbreviation for hexosyl, Hex2 for dihexosyl

Biological samples		Lipid classes	Reference(s)
Cell cultures	HC1*	DG, CL, BMP, PE(+OxPE), PG, PS, LPE, PRs	[69]
	HCC827 (+HCC827-GR)		[109]
	HEK 293	FA, GLs, GPs, PLs, OxGPs, HexCer, CEs, STs	[73]
	HeLa	CL, DG, TG, PC, PE, PG, PI, PS, LPC, LPE, Cer(+HexCer), SM	[70], [130]
	Huh7	LPC, LPE	[92]
	HTC-116		[109]
	Huh7	LPC, LPE	[92]
	MDCK (+MDCK-GR)		[15], [109]
	OVCAR-3		[109]
	OxWR*	CL, DG, BMP, PE, PG, PS, LPE, PRs	[69]
	PANC-1	CE, DG, TG, PC, PE, PI, PS, LPC, Cer, SM	[108]
	PC-9 (+PC-9-GR)		[109]
	PC12	PC, PE, PI, PS	[72]
	RAW 264.7 (macrophages)**	GLs, PA, PC, PE, PG, PI, PS, LPLs, SPs, CEs, cholesterol	[66], [107]
	SKOV-3		[109]
	Bladder cancer cell		[155]
	Melanoma B16***	Cer, SM	[15]
	Primary CD4+T lymphocytes***	Cer, SM	[15]
	Yeast	PA, PC, PE, PG, PI, PS	[140]

*anaerobic, *oxalobacter formigenes* ; **widest lipid range ; ***mouse

Table 10

Table 10. Common lipids according to research topic and identification approach. When “Identified lipids” are indicated as a sum without any explanation, it means that the first number is the amount of lipids found in positive and the second in negative mode (positive+negative).

Citation	Theme	T/reIT/U T		GP's					Hex	Identified lipids	Bio-markers
[47]	Method development	UT	2	1				1		429	
[161]	Method development	relT	1	4				1			
[160]	Method development	UT	2	3	*		2	1	1		
[160]	Method development	UT	2	4	*		2	2			
[160]	Method development	UT		3	*		2	2	1		
[140]	Method development	UT		6	*					436	
[127]	Method development	T/UT	2	6			5	2		207	
[130]	Method development	UT	2	4			2	*		292+206	
[157]	Method development	T	1	4			2	1		22	
[155]	Method development	PT	2	2	*		2	2	2	515+630+640 [D]	
[189]	Method development	UT	2	3			1	2		104	
[159]	Method development	UT								403	
[48]	Method development	UT								85 [E]	
[108]	Method development / cancer research	UT	2	4			2	2		226-414	
[63]	Method development / Metabolomics	UT	*	*						83	8
[46]	Method development / cancer research	T/UT	3	5			3	2	1	132	
[110]	Cancer research	UT	2	1	*			2	1	286	34
[111]	Cancer research	UT	2	2							50

[113]	Cancer research	T/UT	3	5	*			3	2	493	14+10+2 [B]
[92]	Diseases and Biomarkers	UT					2			24	
[101]	Diseases and Biomarkers	UT		4			2	1		129	
[94]	Diseases and Biomarkers	UT	3	6			3	3		749	16
[89]	Diseases and Biomarkers	UT	1	2						12	9
[95]	Diseases and Biomarkers	UT	*	*				*		179	
[95]	Diseases and Biomarkers		*	*				*		196	
[90]	Diseases and Biomarkers	UT	1	2			1	1		746	11
[90]	Diseases and Biomarkers		2	2			1	1			
[91]	Diseases and Biomarkers	UT	3	5				4		261+39	7
[96]	Diseases and Biomarkers	UT	1	2	*		2	2		365	19
[97]	Diseases and Biomarkers	UT	2	2	*		2	2	3	363	28
[98]	Diseases and Biomarkers	UT	1	1						Features	Features
[5]	Diseases and Biomarkers		2	4				1			>25
[102]	Diseases and Biomarkers	relT/T								81 [A]	17 [A]
[103]	Diseases and Biomarkers	UT									77
[9]	Diseases and Biomarkers	UT	2	5			3	2			77
[99]	Diseases and Biomarkers	UT	1	5	*		3	1		188+62	87
[100]	Diseases and Biomarkers		2	1			1	1			
[104]	Diseases and Biomarkers	UT	1	4			1	1		1026	25
[105]	Diseases and Biomarkers	T/UT	2	2	*		1	2		55	55
[106]	Diseases and Biomarkers	UT	1	3	*		2			38+31	11+14
[45]	Drug-testing	UT	1	1			1	1		155	
[56]	Foodstuff profiling	T	1	4			2	1		81	
[67]	Metabolomics	UT	1	5			1	*		61	
[68]	Metabolomics	UT					4				
[62]	Metabolomics	UT	2	4			*				

[65]	Metabolomics	UT	2	5				1		178	
[69]	Metabolomics	UT	1	4	*	1	1			97	
[69]	Metabolomics		1	3	*		1				
[158]	Metabolomics	UT	1	2			2	1		226	
[70]	Metabolomics	UT		5				1		249+451	
[71]	Metabolomics	UT	1	1						7	
[72]	Metabolomics	T		4						22	5
[66]	Metabolomics	UT	3	6			6	7		523	
[73]	Metabolomics	UT	1	7	*	*	5	2	1	642	53
[74]	Metabolomics	UT	2	5	*		4	2	1	611	440
[75]	Metabolomics	UT		6	*		3	2		N/A	≥43
[76]	Metabolomics	T	1	4	*		1	2			126+124 [E]
[87]	Metabolomics	UT		7	*		1	2		119	38
[50]	Physiology	T/UT		*				*		Features	
[58]	Physiology	T									
[15]	Physiology	T						2		45	
[59]	Physiology	UT	1	6	*		2	2		283	
[52]	Physiology	UT								12	
[53]	Physiology	T								5	
[107]	Physiology	UT			*			1		184+150	
[57]	Physiology	UT	2	1							35
[61]	Physiology	UT	3	5			2	1		529+179	

*: Lipid subgroups could not be determined

T/reT/UT: targeted/relatively targeted/untargeted

[A] for hyperlipidemia: plasma(74, 57 biomarkers), VLDL(74, 52 biomarkers), LDL(76, 42 biomarkers), HDL(73, 41 biomarkers)

[B] non-small cell lung cancer+lung benign disease+healthy controls

[C] metabolites

[D] in plasma+tissue+cell

[E]: serum+liver

Table 11. Extension of lipid classes analyzed in different research articles (2017-2019)

[illegible]

[105]	Diseases	T/UT		*	*							55	55
[106]	Diseases	UT	*		*							38+31	11+14
[56]	Foodstuff profiling	T										81	
[67]	Metabolomics	UT	*									61	
[68]	Metabolomics	UT											
[62]	Metabolomics	UT		*									
[65]	Metabolomics	UT			3							178	
[69]	Metabolomics	UT	1			1						97	
[69]	Metabolomics		1			1							
[158]	Metabolomics	UT						*	*			226	
[70]	Metabolomics	UT	1									249+451	
[71]	Metabolomics	UT			1							7	
[72]	Metabolomics	T										22	5
[66]	Metabolomics	UT		*								523	
[73]	Metabolomics	UT	*	*	*				*			642	53
[74]	Metabolomics	UT	2	*								611	440
[75]	Metabolomics	UT	*									N/A	≥43
[76]	Metabolomics	T										126+124	
[87]	Metabolomics	UT										119	38
[50]	Physiology	T/UT	*			*	*	*				Features	
[58]	Physiology	T	*										
[15]	Physiology	T										45	
[59]	Physiology	UT			1							283	
[52]	Physiology	UT			**							12	
[53]	Physiology	T	***									5	
[107]	Physiology	UT	2									184+150	
[57]	Physiology	UT											35
[61]	Physiology	UT	*									529+179	

*Lipid classes not counted

**15 steroids

***5 prostaglandins

[A] for hyperlipidemia: plasma(74, 57 biomarkers), VLDL(74, 52 biomarkers), LDL(76, 42 biomarkers), HDL(73, 41 biomarkers)

[B] non-small cell lung cancer+lung benign disease+healthy controls

[C] metabolites

[D] in plasma+tissue+cell

[E]: serum+liver

Figure 1
[Click here to download high resolution image](#)

Figure 2
[Click here to download high resolution image](#)

Figure 3
[Click here to download high resolution image](#)

Figure 4
[Click here to download high resolution image](#)

Figure 5
[Click here to download high resolution image](#)

Figure 6
[Click here to download high resolution image](#)

Figure 7
[Click here to download high resolution image](#)

Figure 8
[Click here to download high resolution image](#)

Figure 9
[Click here to download high resolution image](#)

Manuscript Number:

Title: Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry

Article Type: Review Article

Keywords: Lipids, liquid chromatography, tandem mass spectrometry; clinical profiling, urine, blood.

Corresponding Author: Professor Heli Marja Marita Siren, Ph.D.

Corresponding Author's Institution: University of Helsinki

First Author: Henri F Avela, MSc

Order of Authors: Henri F Avela, MSc; Heli Marja Marita Siren, Ph.D.

Abstract: Lipids are organic biomolecules, which contribute to energy storing, cellular and subcellular membrane assembly, functionality, signalling, gene expression regulation and surfactant formation. Lipidomics comprises of identification and quantitation of organic lipids, their derivatives and variants. The present review article combines the literature on glycerol-, glycerophospho- and sphingolipids in lipidomics from the years 2017-2019. In addition, a few papers which have promoted advancement in lipidomics are discussed. The primary focus is lipid profiling of biological lipidomic systems with ultrahigh performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS, tandem MS) detection, data handling, and calculations with analytical tools and current statistical methods. Research on in vivo samples includes matrix categories, such as primary blood derivatives (plasma, serum, erythrocytes, and blood platelets), faecal matter, urine, and liver tissues.

Suggested Reviewers: X L Guan Dr
Professor, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
xueli.guan@ntu.edu.sg
Specialists and professional on lipidomics.

Coral Barbas Dr
Professor, CEMBIO, Centre for Metabolomics and Bioanalysis, San Pablo CEU University
cbarbas@ceu.es
Specialist in lipidomics and bioanalysis.

Olivier Berdeaux Dr
Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS, INRA, Université Bourgogne Franche-Comté
olivier.berdeaux@dijon.inra.fr

Specialist in bioanalysis and metabolomics in lipidomics.

Jos F Brouwers Dr
Department of Biochemistry and Cell Biology, Faculty of Veterinary
Medicine, Utrecht University
J.brouwers@uu.nl
Specialist in lipidomics

Cover Letter

To

The Reviews Editor

Helsinki 11.11.2019

Dear Editor,

The paper was informed to be sent to the journal on 16th September, 2019 and asked about considering our review article for publication. The contacted person was Dr. Gregory S Makowski, Reviews Editor, CCA.

Now the review paper is ready and its title is

Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry – A review

written by Henri F. Avela and Heli Sirén

is sent to Clinica Chimica Acta.

For this paper we have compiled the newest literature between 2017 - 2019. The articles reviewed primarily were focused on applications of clinical and medical research made with ultrahigh-performance liquid chromatographic methods with mass spectrometric (MS, tandem MS) detection. The lipid articles were considered to be sufficient enough for making the current statement of the state from glycerol-, glycerophospho- and sphingolipids in lipidomics and the identification with on-line coupled liquid chromatography with mass spectrometry research. Additionally, the data handling with computational methods alongside chemometric and statistical methods are discussed. That kind of evaluation was shown to have increased importance and usage for cross-validation and data-analysis. Please, find the abstract on a separate page.

The authors declare that they do not have competing financial interest concerning the project. They do not have any conflicts, either.

The manuscript has not published in any other journal. It has ten (10) figures and eleven (11) tables.

Thank you for considering the submission. We look forward to your response.

Sincerely

Heli Sirén,

Docent (Assoc.Prof.), University of Helsinki, Finland

Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry – A review

Henri F. Avela and Heli Sirén

Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 University of Helsinki (FI-00560 Helsinki), Finland

*¹ Correspondence: Henri F. Avela (MSc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, Finland; Heli Sirén (Assoc. Professor, Doc), Faculty of Science, Department of Chemistry, University of Helsinki, A.I. Virtasen Aukio 1 (P.O. Box 55), FI-00014 University of Helsinki, Finland, heli.m.siren@helsinki.fi

Abstract

Lipids are organic biomolecules, which contribute to energy storing, cellular and subcellular membrane assembly, functionality, signalling, gene expression regulation and surfactant formation. Lipidomics comprises of identification and quantitation of organic lipids, their derivatives and variants.

The present review article combines the literature on glycerol-, glycerophospho- and sphingolipids in lipidomics from the years 2017-2019. In addition, a few papers which have promoted advancement in lipidomics are discussed. The primary focus is lipid profiling of biological lipidomic systems with ultrahigh performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS, tandem MS) detection, data handling, and calculations with analytical tools and current statistical methods. Research on *in vivo* samples includes matrix categories, such as primary blood derivatives (plasma, serum, erythrocytes, and blood platelets), faecal matter, urine, and liver tissues.

Keywords: Lipids, liquid chromatography, tandem mass spectrometry; clinical profiling, urine, blood.

CCA AUTHOR CHECKLIST

This form is to be submitted with the manuscript. Please fill in and check boxes below.

Article Title: Lipidomics and recent applications in clinical and medical research with ultrahigh performance liquid chromatography-mass spectrometry – A review

Corresponding Author: Henri F. Avela and Heli Sirén

☐ **Structured abstract**

x☒ **Keywords**

x☒ **References are in journal format. References in text and reference list correspond exactly.**

x☒ **Quantities and units conform to international practice**

If this paper is a re-submission, please include:

Article Reference Number:

Figure 10
[Click here to download high resolution image](#)