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Recent advances, challenges, and future directions in the mass spectrometry analysis of glycosphingolipids in biological samples

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ABSTRACT

Mass spectrometry combined with (ultra)high-performance liquid chromatography has proved to be the most suitable method for the analysis of glycosphingolipids (GSLs). An increasing number of studies have focused on the analysis of not only simple but also complex GSLs with the aim of clarifying their biochemical functions in animals and humans. Despite the tremendous improvements in sample preparation and analytical methods in glycosphingolipidomics, there are still several major issues that complicate the comprehensive analysis of GSLs. This review aims to provide the latest advances, developments, and instrumental innovations in the analysis of GSLs in biological samples. The current barriers and challenges facing the qualitative and quantitative analysis of GSLs are highlighted and critically assessed. Overcoming these bottlenecks is crucial for high-throughput GSLs profiling, which is essential to reveal their clinical potential and to understand their role in the pathophysiology of serious diseases, such as cancer.

1. Introduction

Glycosphingolipids (GSLs) are a ubiquitous and remarkably diverse subclass of glycolipids that represent the majority of glycolipids embedded in the cell membrane of virtually all eukaryotes, where they play an important role in many biological and pathophysiological processes. Therefore, GSLs are useful targets for the development of novel diagnostic or prognostic tools and therapeutic methods for a variety of human diseases. GSLs are amphipathic molecules composed of a hydrophilic glycan head group and a hydrophobic ceramide backbone mutually linked via a glycosidic linkage. The ceramide part is composed of a sphingoid base and an N-linked fatty acyl chain [1] (Fig. 1). The biosynthesis of GSLs proceeds in a step-by-step fashion, initiated first by ceramide synthesis followed by the addition of a carbohydrate unit to the ceramide and then to the growing glycan chain (elongation or branching). The synthesis of GSLs is tightly regulated by the ordered action of specific enzymes called glycosyltransferases that produce an astonishing number of structurally distinct molecular species with >500 glycans characterized to date [1–3]. GSLs are commonly classified into subgroups of series according to the different core structures and physicochemical properties of their glycan chains [4]. Due to the immense structural variability derived from the heterogeneity in both glycan and ceramide moieties, a comprehensive and uniform nomenclature is crucial for the unambiguous interpretation of the GSLs. It is recommended to follow the IUPAC nomenclature [5], which is adopted by the Lipid Metabolites and Pathways Strategy consortium (LIPID MAPS) [6], along with updated versions published by Liebisch et al. [7]. Nevertheless, this nomenclature is not practical for more complex GSLs, which are easier to understand from graphical representations using glycan symbols according to the widely accepted Symbol Nomenclature for Glycans [8]. In addition to being the key membrane components, GSLs are bioactive effectors with many other intriguing and versatile functions that are determined collectively by the glycan and lipid parts. The main biological functions of GSLs have been reviewed elsewhere [9–11]. Over the past decades, the altered metabolism of GSLs caused primarily by the gene expression dysregulation of glycosyltransferases has been shown as one of the hallmarks closely associated with the pathogenesis of various diseases, such as lysosomal storage disorders (LSDs) characterized by the accumulation of specific GSL, neurodegenerative diseases, where changes in GSL, specifically gangliosides, have also been described [12], autoimmune diseases, such as peripheral neuropathies [13], infectious diseases, where they act as binding sites or receptors for pathogens and their toxins, diabetes, cardiovascular diseases [14] and in various types of cancers [15,16]. Although the

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involvement of GSLs in the onset and/or progression of various diseases and cancers has been reviewed several times in the last 5 years [17–19], none of these reviews has yet provided a comprehensive overview that takes into account not only simple GSLs, but also the vast majority of more complex sialylated and/or fucosylated GSLs.

2. Recent advances and trends in analytical methods in glycosphingolipidomics

In recent years, the analysis of GSLs has seen notable advances and evolving trends driven by the intersection of cutting-edge technologies and the growing recognition of the biological significance of these complex biomolecules. Specifically, (ultra)high-performance liquid chromatography ((U)HPLC) using sub-2-μm particles has increasingly replaced conventional HPLC due to improved separation efficiency and resolution together with reduced analysis time that allows high-throughput analyses [20]. Lipid class separation using hydrophilic liquid chromatography (HILIC) and reversed-phase HPLC for lipid species separation are the most powerful and frequently employed chromatographic modes for GSL analysis. Mass spectrometry (MS) is used most frequently for the detection of separated GSLs. Other less common separation techniques, such as capillary electrophoresis, can be used for GSL analysis or glycan profiling [21,22]. Atmospheric pressure ionization (predominantly electrospray ionization (ESI) and occasionally atmospheric pressure chemical ionization) and ambient ionization methods (mainly matrix-assisted laser desorption/ionization, MALDI and desorption electrospray ionization, DESI) have become pivotal for GSLs analysis [23,24]. Moreover, ultrahigh-performance supercritical fluid chromatography (UHPSFC)/MS [25] or even 2D SFC/LC/MS [26] have emerged as promising alternatives to HILIC/MS offering improved lipid coverage, selectivity, sensitivity, and reduced analysis time. However, the application of SFC in glycosphingolipidomics remains very limited. Recently, ion mobility (IM) has seen significant improvements and gained a great deal of attraction in glycosphingolipidomics by providing an additional dimension of separation. Although IM technologies have evolved greatly, isomer separation remains very challenging as only a few studies have successfully applied IM for GSL isomer separation [27,28], for example, GD1a/GD1b, GlcCer/GalCer and GlcSph/GalSph [29–31]. However, continuous innovation and recent developments in IM have made its popularity increase by improving both the separation power of isomers (selectivity) and significantly reducing chemical noise, allowing the analysis of rare and low abundant GSLs (sensitivity) [27,32]. Moreover, changing experimental variables (*e.g.,* pressure, temperature, and composition of the gas, or induction of gas additives) can lead to ion mobility shifts and thus significantly affect the separation efficiency [27]. Various IM modes have been reported for the isomeric separation of GSLs, such as trapped ion mobility spectrometry (TIMS) [31], or structures for lossless ion manipulation (SLIM) technology [33]. Additionally, ^{1}H nuclear magnetic resonance (NMR) is a complementary method for the structural determination of GSLs providing additional information despite being less used and less sensitive than LC/MS-based methods [34]. Advanced multidimensional NMR techniques can provide enhanced resolution and sensitivity, which are particularly effective in resolving overlapping signals and capturing fine details of glycan sequences and lipid moieties. ^{13}C and ^{15}N NMR facilitate the assignment of specific resonances of GSLs, enabling more accurate structural determination and differentiation of glycan isomers [34,35].

3. Current obstacles facing qualitative and quantitative analysis of GSLs

3.1. Sample pretreatment

The preanalytical steps, such as sample collection, handling, and storage, are the first and most critical steps that must be performed thoroughly to maintain sample integrity and minimize analytical errors. Indeed, a large number of laboratory errors may arise from these preanalytical steps [36]. A detailed description of various preanalytical factors affecting the stability of lipids in biological samples has recently been thoroughly reviewed [37,38] together with the summary of advantages and disadvantages of pretreatment strategies and storage conditions used in lipidomics. Furthermore, Reis et al. [39] investigated the stability of lipids in human plasma and serum with respect to temperature-related storage conditions and noted that analytical biases can arise even when samples are stored at −80 °C, especially when stored for more years. These changes were observed in particular for nonpolar lipids. Furthermore, Wolrab et al. [40] determined the one-year stability of lipids in human plasma and serum, reporting that specifically glycerolipids were prone to change over time. Ulmer et al. [41] described various factors that affect lipid stability (*e.g.,* chemical oxidation, enzymatic processes, or thermal decomposition) and

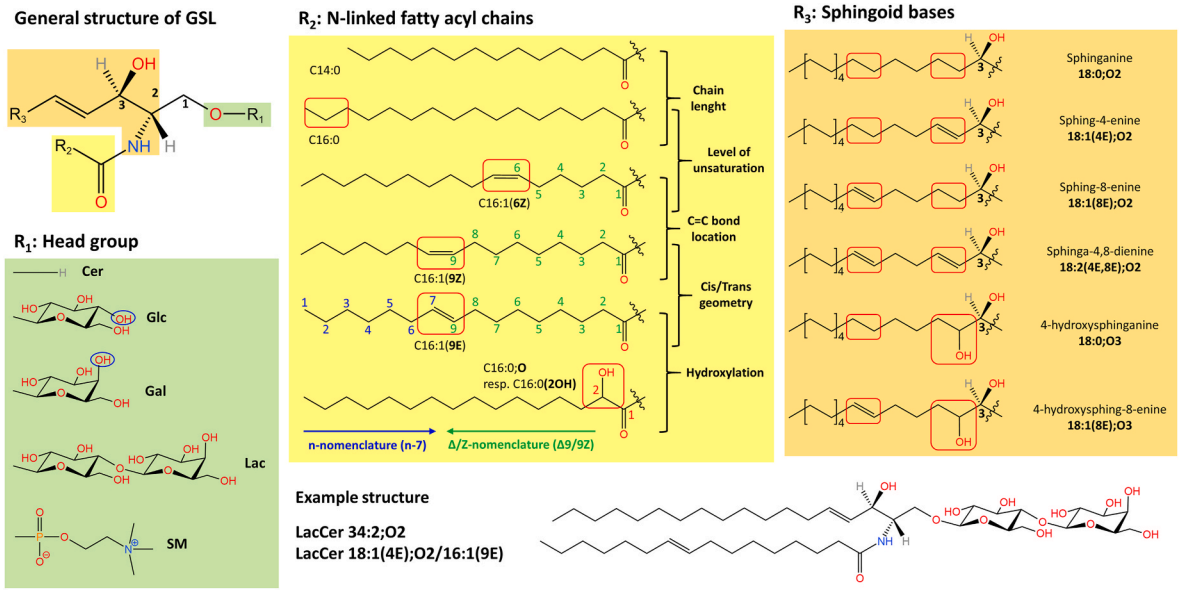

Fig. 1. Overview of typical glycosphingolipid structures (namely, LacCer 36:1; O2, resp. 18:1; O2/18:0), including possible modifications in both hydrophilic and hydrophobic parts.

suggested recommendations for ensuring lipid stability during sample pretreatment. Ideally, only fresh samples should be used. However, the majority of samples are collected and analyzed at different locations, making sample storage prior to analysis virtually inevitable. Consequently, samples should be processed as quickly as possible and then flash frozen with liquid nitrogen and stored at −80 °C to minimize unwanted processes [38]. Hammad et al. [42] examined the impact of sample collection methodology using various anticoagulants and reported that EDTA tubes provide the least variability in GSL levels compared to citrate and heparin tubes. In contrast, Wolrab et al. [40] also compared collection tubes, but did not include any GSL subclasses. Thus, EDTA should be the preferred anticoagulant for the lipidomic analysis including GSLs. A crucial matter in lipidomics is primarily the high susceptibility of unsaturated fatty acids to variable chemical reactions (*e.g.,* oxidation), a major source of lipid degradation. However, GSLs are considered more resistant to oxidation since their ceramide backbones are generally more saturated [38]. Nonetheless, the literature still lacks comprehensive studies dealing with the factors affecting GSL stability along with very little available data on specific storage conditions and handling protocols.

3.2. Extraction and purification strategies

Another critical step for the successful analysis of GSLs is their efficient extraction from highly complex biological matrices using appropriate solvent mixtures along with the removal of interfering substances.

3.2.1. Extraction

The chloroform-methanol based extraction protocols introduced by Folch [43] and Bligh-Dyer [44] in the 1950s have been the gold standard in lipidomics for decades. Recently, various modified methods have been developed to effectively extract a wide range of lipids. Matyash et al. [45] established a method based on methyl-tert-butyl ether and methanol. Furthermore, butanol-methanol method was described by Löfgren et al. [46] and further modified by Alshehry et al. [47]. Recently, Vale et al. [48] employed a novel single-step three-phase extraction system for the successful extraction of neutral lipids and phospholipids. Most recently, Höring et al. [49] compared one-phase extractions using methanol, ethanol, acetonitrile, acetone, isopropanol, butanol as well as their mixtures and reflected that their application should be limited to polar lipids for which provide high recovery (>90 %) and reproducibility (<20 %), or should be further followed by sample clean-up. Although these protocols provide high recovery for a wide range of lipids, they are not so effective for GSLs. Teo et al. [50] reported that Matyash's method was able to recover only 10 % of the main polar lipids, including glycolipids. The abundances of GSLs in biological samples vary and specifically more complex GSLs are low abundant [51,52], as also demonstrated in our recent works [53,54]. Thus, the extraction of a wide range of GSLs must take into account their specific physicochemical properties and generally requires more specialized or multistep extraction methods [55], such as that of Karlsson [56], which utilizes multiple purification and sub-fractionation steps to isolate even more complex GSLs. Although this method was introduced more than 30 years ago, it remains a convenient method for the extraction and purification of complex and low-abundance GSLs. However, it is too time-consuming and laborious [23,55]. Unfortunately, no rapid and efficient extraction method capable of uniformly extracting a wide range of GSLs in high yields and allowing high-throughput screening is available. This is primarily caused by a wide range of polarities within GSL subclasses. For instance, highly polar GSLs (*e.g.,* gangliosides and sulfatides) or neutral GSLs with more than four monosaccharide residues largely partition into the methanol-rich layer, while less polar GSLs (*e.g.,* GlcCer, GalCer, or LacCer) tend to remain in the chloroform-rich layer. It should also be highlighted that a large part of specifically more complex gangliosides may be lost when analyzing only one layer [57,58], and therefore, ganglioside extraction may require additional purification and fractionation by SPE [57]. These obstacles in the extraction of GSLs are mainly due to their structural heterogeneity that covers different polarities and different chemical stability [55]. Therefore, the development of rapid and sophisticated extraction methods that allow simultaneous isolation of both hydrophobic and hydrophilic GSLs is essential to enable the high-throughput analysis of GSLs. There are also general concerns about the effects of toxic solvents on human health and the environment, which reinforce efforts to transition towards green chemistry by implementing alternatives to chlorinated solvents [59].

3.2.2. Purification

The removal of present highly abundant lipids (*i.e.,* phospholipids, acylglycerols, and sphingomyelins) together with other unwanted compounds (*e.g.,* proteins and salts) from biological samples is strongly recommended because GSLs are minor components [55]. Specifically, the removal of phospholipids represents a notable challenge required to reduce matrix effects and increase the ionization efficiency of GSLs [38,55]. Alkaline hydrolysis has been shown to be suitable for the elimination of major phospholipids and acylglycerols, while GSLs are relatively resistant compared to other ester-linked lipids [55]. GSLs can then be further purified and fractionated from alkali-labile and alkali-stable phospholipids and other contaminants using silica-based chromatography together with weak anion-exchange chromatography using diethylaminoethyl-linked matrices, which is particularly advantageous for the separation of neutral and acidic GSLs [52,54,55]. However, it should be noted that O-acetylated derivatives also degrade [60]. Alternatively, solid-phase extraction (SPE) can be successfully applied for isolation, purification, and enrichment of minor lipid subclasses using either normal-phase (silica), reversed-phase (C8/C18), or ion-exchange (aminopropyl) columns [59]. Newly synthesized ZrO_2/TiO_2-coated sorbents [61] allow selective isolation of GSLs from phospholipids and sphingomyelins without the use of alkaline hydrolysis [62,63]. Moreover, sulfobetaine-based zwitterionic sorbents have also been shown to selectively retain phospholipids [64]. SPE can be applied in combination with common liquid-liquid extraction as an additional cleaning step [53,65], but the selectivity of SPE is limited and can result in the adsorption of matrix components. In summary, there are still two major challenges to overcome, namely, (1) the rapid and uniform extraction of a wide range of GSLs and (2) the complete removal of highly abundant interfering and unwanted substances.

3.3. Qualitative and quantitative analysis

3.3.1. Separation of isomers

One of the current major challenges is the difficulty in separating isomeric and/or isobaric species as a result of the immense structural variability of lipids. Consequently, the specific functions of isomers have remained largely unknown [66]. This problem is multiplied in the case of GSLs because many glycans have the same formula (Glc *vs.* Gal) [67]. Although MS is a very selective method, isomers often provide the same or very similar fragmentation. Hence, the identification and differentiation of isomers in complex samples requires coupling with (U)HPLC, IM, and/or alternative methods, such as novel specific fragmentation approaches or chemoselective derivatization that provide diagnostic ions of each lipid species. Moreover, specific glycan-detaching enzymes, such as endoglycoceramidase (EGCase) or ceramide glycanase (CGase) in combination with MS/MS analysis, have been shown to be advantageous for the differentiation of isomers by providing information about the linkage position-based on the specific cross-ring and double-cleavage fragments, together with the enhanced ionization. EGCase I is the best glycan-detaching enzyme with a broad substrate specificity that is well-suited for GSL analysis [68].

Specific chromatographic techniques have previously been applied to separate lipids according to the number and positions of double bonds (silver-ion HPLC) [69] or *sn*-positions (chiral HPLC) [70]. Recently,

Vaňková et al. [71] demonstrated a highly reliable UHPLC/MS method capable of identifying several hundreds of lipids within a wide range of polarities with significantly reduced run time. Remarkable separation power for GSL-derived oligosaccharides and isomers using porous graphitized carbon (PGC) columns has been reported by Teneberg [52,54] and Wuhrer [72] groups. Mank et al. [73] used Hypercarb PGC column to separate and characterize human milk oligosaccharides by LC/ESI-MS^2 along with Cho et al. [74], who investigated the separation of permethylated glycans using nanoflow LC/MS. In recent years, IM has been shown to be a powerful technique for the separation of lipid isomers. Wojcik et al. [66] achieved a partial separation of GlcSph/GalSph 18:1; O2 and GlcCer/GalCer 18:1; O2/18:0 after four passes (~60 m path) using IM separation with multipass SLIM platform. The baseline separation of GD1a/GD1b gangliosides was achieved even with a minimal possible path of 1.25 m. May et al. [75] resolved GD1a/GD1b gangliosides in a standard mixture together with two pentasaccharides differing in the location and linkage of fucose. Djambazova et al. [31] also reported a partial separation of GD1a/GD1b isomers with 36:1; O2 and 38:1; O2 ceramide in tissue samples using MALDI-TIMS. Additionally, Xu et al. [76] showed effective resolution of GlcCer and GalCer species from human plasma and cerebrospinal fluids using differential mobility spectrometry coupled to LC/ESI-MS. In summary, only a limited number of studies have been able to achieve baseline separation of GSLs isomers even when using cutting-edge MS or IM instruments. The separation of isomers is still a challenging task. Consequently, more progress is needed as potential applications of the evolving IM are still being discovered [28].

3.3.2. *Derivatization strategies*

Recently, ambient ionization MS coupled with novel ion activation technologies, such as Paternò-Büchi reaction [77], ozone-induced dissociation [78], epoxidation [79], light-controlled photoepoxidation [80], electrochemical epoxidation [81], chloroauric acid-dopped solvent-induced epoxidation [82], low-temperature plasma-induced epoxidation [83], and selective photosensitized oxidation induced by singlet oxygen [84], have yielded more informative and abundant specific fragment ions allowing rapid and direct structural elucidation of double bonds, *cis/trans* isomers, and *sn*-position isomers of GSLs and other lipids [85]. The principles of these methods are well described in other reviews [86,87]. Additionally, a novel airflow-assisted DESI method has been proposed to conveniently characterize the spatial distribution of unsaturated lipid isomers using MSI [88]. GSLs are typically low abundant and suffer from low ionization efficiency, particularly neutral GSLs and those heavily glycosylated. Therefore, the derivatization can significantly increase the ionization efficiency and sensitivity [38,89]. The principles of main derivatization strategies are summarized and illustrated in Fig. 2. The most common derivatization reaction is permethylation, where the active protons in –OH and $–NH_2$ groups of GSL molecules are substituted with the methyl group using natural ($^{12}CH_3I$) and/or isotopically-labelled methyl iodide ($^{13}CH_3I$ or $^{12}CD_3I$) [55]. The observed unique fragments enable the assignment of glycan sequences including linkages. Consequently, permethylation in combination with UHPLC/MS or IM has great potential to overcome existing analytical barriers in the separation and differentiation of isomers [89]. Furthermore, alkaline conditions during permethylation degrade ester-linked lipids such as highly abundant glycerolipids and glycerophospholipids, which is advantageous for the detection of low abundant GSL due to reduced ion suppression [90]. Unfortunately, even mild alkaline conditions during permethylation will also likely lead to the loss of pH-sensitive GSLs (O-acetylated or polysialylated GSLs),

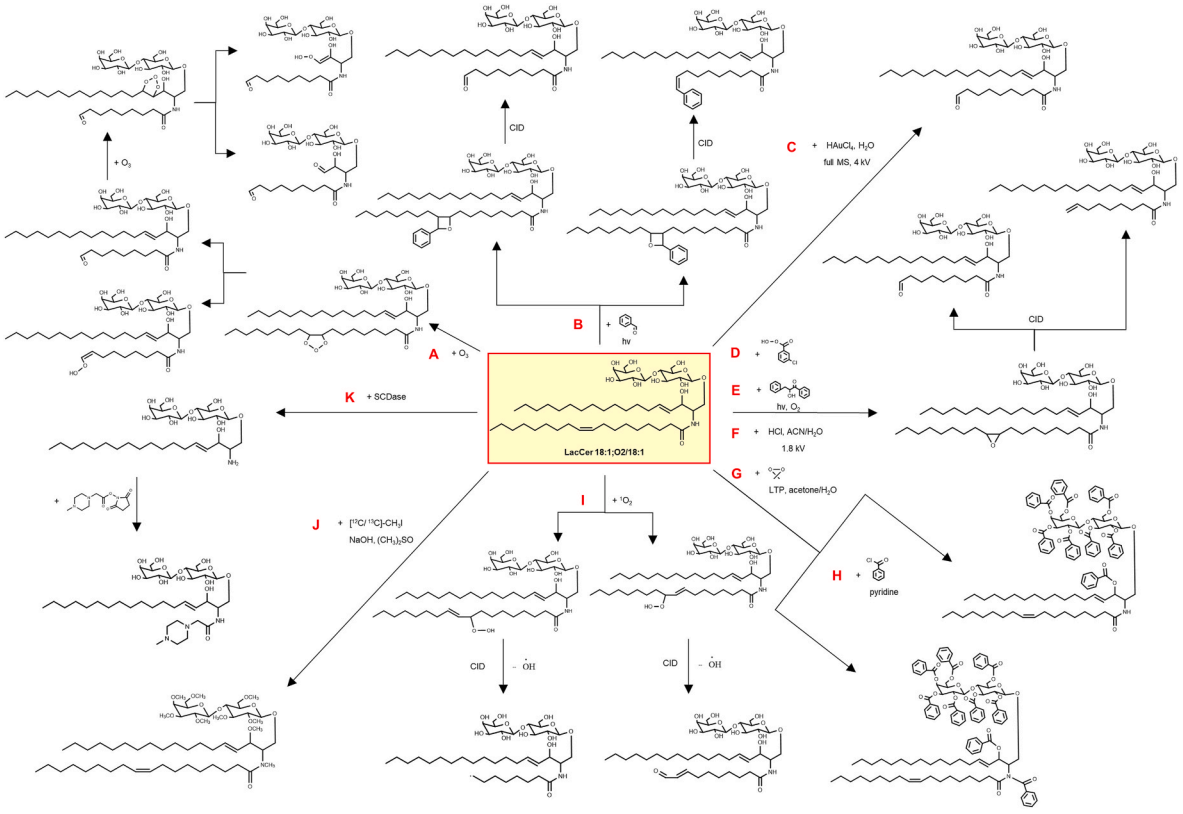

Fig. 2. Derivatization strategies used for the structural elucidation of GSLs on the example of LacCer 18:1; O2/18:1. A/ozone-induced dissociation [78], B/Paternò-Büchi reaction [77], C/chloroauric acid-doped solvent-induced epoxidation [82], D/mCPBA epoxidation for lipid double-bond identification (MELDI) [143], E/light-controlled photoepoxidation [80], F/electrochemical epoxidation [81], G/low-temperature plasma-induced epoxidation [83], H/derivatization using benzoyl chloride [96], I/selective photosensitized oxidation induced a singlet oxygen [84], J/isotopic permethylation [55], and K/isobaric labelling using tag iTRAQ™ [55,95].

which is undesired [91]. Generally, gangliosides are known to be prone to loss of sialic acid during in-source and post-source dissociation, which decreases the sensitivity of molecular ions, the major problem especially in MALDI-MS [92]. Consequently, methyl esterification, amidation, permethylation, or perbenzoylation methods have been developed to stabilize the sialylated residues and to improve the ionization efficiency of acidic GSLs [93]. The discrimination between α2,3 and α2,8-linked sialic acid isomers can be made by ring-opening aminolysis [94]. In addition, the carboxyl group of sialic acid labelled with an easily ionizable tertiary amine N,N-dimethylethylenediamine [93] has enhanced the ionization and provided diagnostic ions that facilitate the discrimination of ganglioside isomers. Another relatively novel derivatization strategy is isobaric labelling using commercial products, such as isobaric tag for relative and absolute quantitation (iTRAQ™) reacting with free amines and aminoxy tandem mass tag (aminoxyTMT™) reacting with carbonyls [55,95]. Recently, Peterka et al. [96] have introduced a simple, robust, and highly reproducible derivatization of multiple lipid subclasses, including simple GSLs, using non-hazardous benzoyl chloride. There are also several other strategies used for the structural elucidation of GSLs, such as chemical oxidation using diluted $NaIO_4$, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, or oxidative release of natural glycans [55].

3.3.3. *Quantitation*

The quantitative analysis in lipidomics is a multifaceted endeavor to determine the relative or absolute amount of lipids in the sample [97] and relies on the whole lipidomics workflow (Fig. 3), including rigorous validation and careful selection of ISs [19]. Nonetheless, achieving broad-based absolute quantification is challenging due to a large number of estimated lipid species, ranging from 10,000 to 100,000, with concentration levels varying significantly between biological samples together with a lack of intra- and interlaboratory reproducibility [98]. The reliable quantitation of GSLs is primarily limited by the lack of suitable and commercially available ISs, especially for more complex

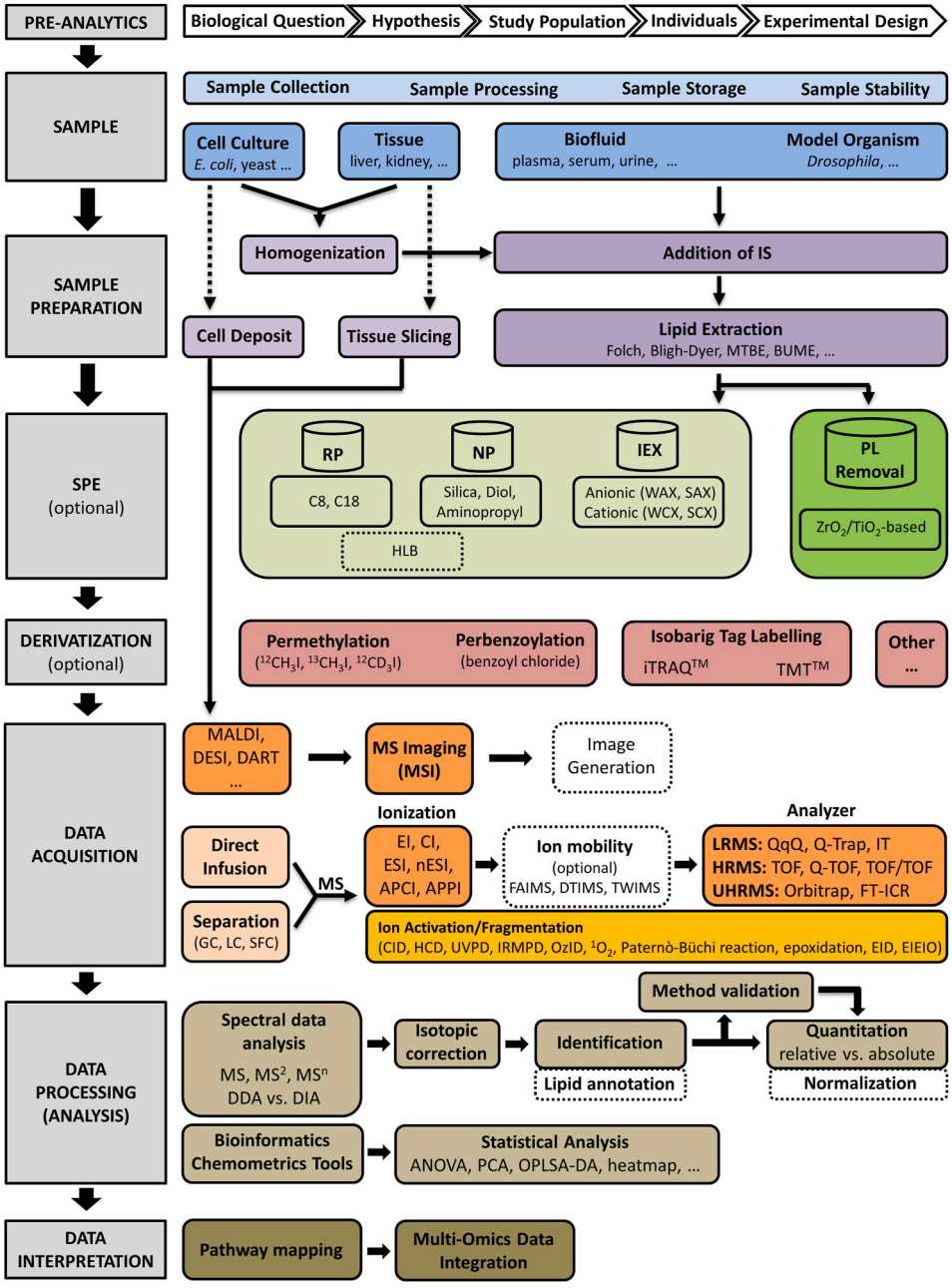

Fig. 3. Comprehensive scheme of all steps applicable in the characterization of GSLs.

GSLs. Generally, at least one exogenous IS per each lipid subclass should be used, but preferably multiple isotope-labelled IS covering the whole lipid subclass should be used to compensate for variability in lipid structures [97,99]. Most ISs are of exogenous origin and are isotopically labelled analogues of endogenous GSLs or have atypical fatty acyl chains (shorter or odd carbon number) fatty acyl chains [100] (Table 1).

Table 1
Commercially available standards for GSL analysis (as of November 2023).

GSLs subclass	Endogenous standards	Exogenous standards	Isotopically labelled internal standards
GalCer	18:1; O2/16:0[a,b,c]	18:1; O2/8:0[a,b,c]	18:1-d_7;O2/13:0[a]
	18:1; O2/18:0[a]	18:1; O2/12:0[a,b,c]	18:1-d_7;O2/24:1[a]
	18:1; O2/18:0; O[a]	18:1; O2/15:0[b,c]	18:1; O2/22:0-d_4[b,c]
	18:1; O2/18:1[a]		
	18:1; O2/22:0[b,c]		18:1; O2/18:0-d_{35}[b,c]
	18:1; O2/24:0[a]		
	18:1; O2/24:1[a]		
GlcCer	18:1; O2/16:0[a]	18:2; O2/6:0[b,c]	18:1-d_7;O2/15:0[a]
	18:1; O2/18:0[a]	18:1; O2/8:0[a]	18:1-d_5;O2/18:0[a]
	18:1; O2/18:1[a]	18:1; O2/12:0[a]	18:1-d_5;O2/18:1[a]
	18:1; O2/22:0[b,c]	18:1; O2/17:0[a]	18:1; O2/16:0-d_3[b,c]
	18:1; O2/24:1[a]		18:1; O2/22:0-d_4[b,c]
LacCer	18:1; O2/16:0[a,b,c]	18:1; O2/8:0[a]	18:1-d_7;O2/15:0[a]
	18:1; O2/18:0[a,c]	18:1; O2/12:0[a]	18:1-d_7;O2/24:1[a]
	18:1; O2/18:1[a]	18:1; O2/17:0[a,b,c]	18:1; O2/16:0-d_3[b,c]
	18:1; O2/24:0[a]		
	18:1; O2/24:1[a]		
Gal_2Cer		18:1; O2/17:0[a]	
(i)Gb_3Cer	18:1; O2/16:0[b,c]	18:1; O2/17:0[a,b,c]	18:1; O2/18:0-d_3[b,c]
	18:1; O2/18:0[b,c]	18:1; O2/17:0[a] (i) 18:1; O2/23:0[b,c]	18:1; O2/16:0-d_9[c]
(i)Gb_4Cer			
SGalCer	18:1; O2/16:0[b,c]	18:1; O2/2:0[c]	18:1-d_7;O2/13:0[a]
	18:1; O2/18:0[b,c]	18:1; O2/12:0[a,b,c]	18:1-d_7;O2/24:1[a]
	18:1; O2/18:0; O[a]	18:1; O2/17:0[a,b,c]	18:1; O2/18:0-d_3[b,c]
	18:1; O2/18:1[b,c]	18:1; O2/19:0[a,b,c]	
	18:1; O2/24:0[a,b,c]		
	18:1; O2/24:1[a,b,c]		
SLacCer	18:1; O2/18:0[b]		
GM_1	18:1; O2/16:0[b,c]	18:1; O2/17:0[a]	18:1; O2/18:0-d_5[a]
	18:1; O2/18:0[b,c]		18:1; O2/18:0-d_3[b,c]
	18:1; O2/20:0[a]		18:1; O2/16:0-d_9[b,c]
	18:1; O2/24:1[b,c]		18:1; O2/24:1-d_{18}[b,c]
GM_2	18:1; O2/18:0[a,b]		18:1; O2/18:0-d_3[b,c]
	18:1; O2/24:1[b,c]		18:1; O2/16:0-d_9[b,c]
GM_3	18:1; O2/18:0[a]		18:1; O2/16:0-d_9[b,c]
			18:1; O2/18:0-d_5[a]
			18:1; O2/18:0-d_3[b,c]
GD_1	18:1; O2/18:0[a] (GD_{1a})	18:1; O2/17:0[a] (GD_{1a})	
	18:1; O2/18:0[a] (GD_{1b})		
GD_2			
GD_3	18:1; O2/18:0[a]		18:1; O2/18:0-d_3[b,c] (GD_3)
GT_1	18:1; O2/18:0[a] (GT_{1b})		
GT_2			
GT_3			
GQ_1	18:1; O2/18:0[b] (GQ_{1b})		

The list of commercially available standards for GSL analysis is based on three major companies offering standards for GSL analysis. Therefore, the list may not be necessarily complete as other companies may offer other standards as well.

[a] Avanti Polar Lipids, Alabaster, AL, USA, www.avantilipids.com.
[b] Matreya LLC, State College, PA, USA, https://www.matreya.com.
[c] Cayman Chemicals Ann Arbor, MI, USA, https://www.caymanchem.com.

^{13}C-labelled ISs are the most convenient due to the identical retention behavior and impossible migration of labelled atoms during fragmentation unlike to deuterium, but they are expensive and mostly not commonly available [101]. This scarcity requires the exploration of alternative strategies, such as *in-house* chemoenzymatic [102,103] or stable isotope-labelled synthesis of GSLs, including the incorporation of isotopically labelled atoms, *e.g.,* ^{2}H, ^{13}C or ^{15}N, into the lipid structure [104]. The possible use of the ^{13}C-labelling approach for all lipids, including GSLs, has gained attraction as a cost-effective and practical solution for reliable quantitation and has recently been deeply investigated *in vivo* by Jaber et al. [101] and well-reviewed by Rampler et al. [105]. Nevertheless, the fully labelled mammalian organism is still missing [105]. The ^{13}C-labelling approach using a stable isotope-labelled substrate (*e.g.,* ^{13}C-glucose), which is metabolized by a biological system and incorporated into the metabolites, would fully compensate for different matrix effects and variations in ionization efficiency, allowing more accurate and precise quantitation [98,106]. Such generated isotope-labelled molecules can also be used as tracers in lipidomics for pathway mapping to reveal the fate of isotope-labelled precursors [106]. The important aspects and considerations of the use of tracers to study lipid flux are summarized elsewhere [107]. The differential isotope labelling ^{12}C/^{13}C permethylation strategy can also be used for accurate relative quantitation [90]. In addition, chromatogram binding assays should also be performed in combination with MS data in cases, as these assays can accurately reflect both the relative amounts of GSLs and possible alterations between the studied samples, but care must be taken due to cross-reactivity of some antibodies [52]. Another issue is that solid materials (*e.g.*, tissues) may trap ISs and thus potentially increasing the variation in results and impairing quantitation. Furthermore, the limited availability or absence of well-defined reference materials (*e.g.,* NIST SRM 1950) complicate reliable quantification and further hamper method validation together with intra- and/or inter-laboratory comparisons [98,108]. IS mixtures of deuterated analogues have been designed and prepared to simplify and mimic the endogenous lipid subclass distribution in a particular sample matrix [98,100]. For example, the series of SPLASH LIPIDOMIX and UltimateSPLASH standards have been designed and designed for the quantitative analysis of human plasma [98].

3.3.4. *Method validation and harmonization*

Method validation is a crucial aspect to ensure that the method is suitable for the given purpose and provides reliable analytical results, which is key to the applicability of the method in clinical trials [25]. Various organizations, such as FDA, EMA, and ICH, developed guidelines for the validation of bioanalytical methods. Nonetheless, method validation across different laboratories is often incomplete or lacking, which together with missing harmonized protocols, including the whole lipidomic workflow makes the comparison of results between studies challenging [109,110]. The main sources of unwanted variations that cover the whole lipidomics workflow have recently been systematically and thoroughly reviewed [111]. The lipidomics community driven by ILS and LIPID MAPS has recently initiated significant efforts towards harmonization and standardization of lipidomic workflows to increase transparency and address discrepancies in published results across different laboratories [112,113]. Nevertheless, common issues, such as incorrect annotation, misidentification, and overreporting, as pointed out by Köfeler et al. [114], along with disparities in lipid concentrations leading to incorrect biological interpretations, will continue until standardized guidelines have been published and implemented [113]. Consequently, harmonization and standardization in lipidomics are highly demanded to assure high standards of data quality and to improve the comparability of lipidomic results obtained with different methods in various laboratories [108,112]. The interlaboratory comparison is of paramount importance in lipidomics due to the lack of suitable reference materials and is crucial for translating lipidomics into clinical practice [98,115].

3.4. Data processing and software tools

Over the past few years, advances in MS-based techniques have allowed considerable progress in lipidomic analysis, enabling researchers to routinely identify hundreds of unique lipids in various biological samples [116]. This is evidenced by an ever-increasing number of lipidomic studies generating extremely large datasets that need to be processed. However, manual processing is time-consuming and requires sophisticated solutions to support automation [117]. To address this issue, numerous open-source software packages have been developed and tailored to enable effective computational processing of data [117], however, most of them have strict formatting requirements that do not allow data processing from all MS platforms [118]. The most widespread software tools tailored specifically for GSL are GlycoWorkbench [119], GlycoPattern [120], GlycoDigest [121], and Mass Spectrometry Analysis Tools within Lipid MAPS (https://www.lipidmaps.org/resources/tools/ms). Moreover, current software tools face the challenge of keeping up with rapid technological progress [122]. Therefore, there is an urgent need for an appropriate update of existing software and the development of novel and comprehensive open-source bioinformatics tools to capture data and facilitate its automated processing [116,118]. Numerous freely available bioinformatic tools used in lipidomics have been summarized in several recent works [20,23,117] along with an excellent review by Hoffmann et al. [122]. Moreover, Ni et al. [117] provided guidance on the appropriate selection of software tools for lipidomic applications and evaluated data processing pipelines. Recently, Lin et al. [118] developed a highly flexible and user-friendly web-based platform called LipidSeg designed for efficient data mining to help users identify important lipid-related features. Furthermore, Ross et al. [123] provided an evaluation of software tools for IM technologies that are currently evolving and highlighted the still restricted adoption of IM including the lack of software support.

4. Translation into clinical practice

Lipidomics has enormous potential for monitoring the prognosis, diagnosis, and treatment of various diseases, including cancer, based on disease-specific biomarkers obtained ideally noninvasively from body fluids [124]. Multiomics interconnection is the key to understanding the molecular mechanisms of diseases to further promote the discovery of disease-specific biomarkers [125,126]. To date, several studies have shown highly promising results, suggesting several GSLs as diagnostic or prognostic markers [52,54,65,127,128]. Specifically, various GSLs have been several times shown to be implicated in various glycosphingolipidoses, which are also termed lysosomal storage disorders, including Gaucher, Krabbe, Fabry, Farber, Sandhoff, Tay-Sachs, and Niemann-Pick diseases as well as metachromic leukodystrophy (see Fig. 4) [12,129]. The specific production of ganglioside antibodies has also been found in a variety of autoimmune diseases, including Guillain-Barré and Miller-Fischer syndromes and a few other neuropathies [13,130,131]. However, none of them has yet undergone prospective clinical trials and has not been translated into clinical practice [132]. Clinical translation represents a completely new challenge due to two major issues: (1) the absence of large population-based studies and (2) the lack of standardized and harmonized analytical workflows [109,133]. Although some studies have shown low intra- and inter-instrument variability of results [108,134], the aforementioned obstacles represent a current barrier to the deployment of these methods in clinical settings. Blood-based cancer early detection tests have emerged as a promising noninvasive screening tool in recent years [127,135–137], however, GSLs are rarely included or completely missing. Moreover, the absence of large-scale validation studies and limited lipid coverage prevent their implementation into population-based screening programs and translation into routine clinical practice [138]. Multicancer detection assays validated on human samples with various strategies currently under development were extensively reviewed by Brito-Rocha et al. [138], but most of them are DNA/RNA-based tests. The dried blood spot analysis is an alternative noninvasive method tested in various omics approaches, including lipidomics [139] together with other dried matrix microsampling methods, such as dried plasma or serum spots, dried blood spheroid, volumetric tip, capillary or tube microsampling methods may be used as well [140]. Additionally, monitoring of lipid flux via a stable isotope labelling approach can be

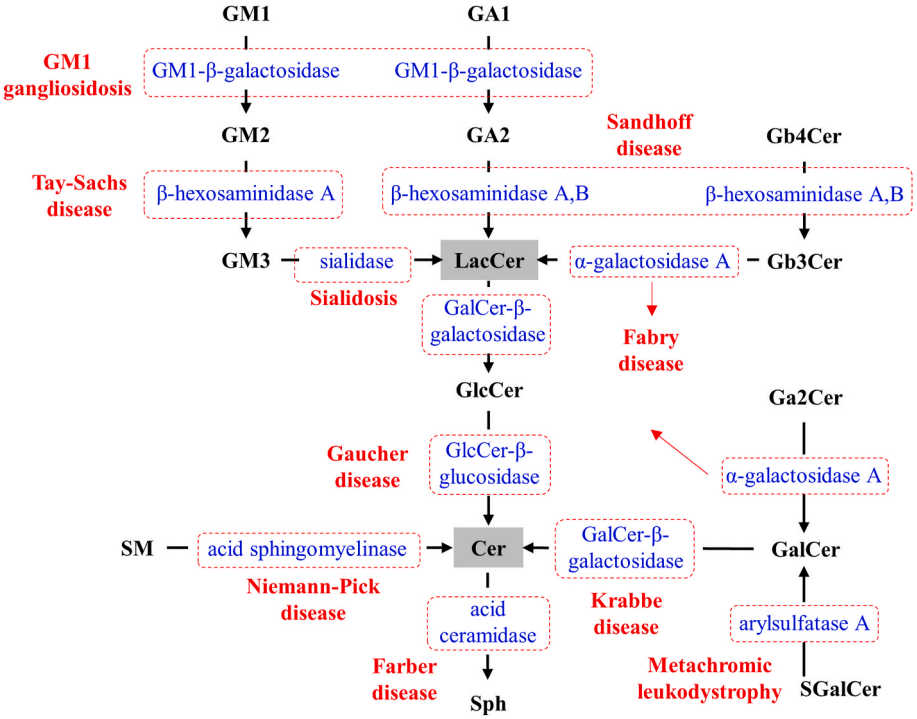

Fig. 4. Schematic illustration of lysosomal storage disorders (LSDs) [12,129].

used to track the real-time distribution of GSLs and other lipids *in vivo* to reveal their roles in pathophysiology [107]. Notable global efforts to tackle lipidomics translation into clinical laboratories for diagnostic and therapeutic purposes are driven by the lipidomic community, including the LIPID MAPS and the Lipidomics Standards Initiative [141]. Readers who are more interested in translating lipidomics into clinical practice are encouraged to read the following reviews [132,142].

5. Conclusions and future perspectives

The rapid progress in MS-based techniques and the development of novel sample preparation workflows have recently had a tremendous impact and significantly boosted the evolution of glycosphingolipidomics. Nevertheless, several challenges must be overcome to realize the full potential of glycosphingolipidomics and allow its translation into clinical practice in the foreseeable future. The major key issues are: (1) the urgency of a fast and effective isolation strategy able to extract structurally diverse and minor GSLs, (2) the separation of GSLs isomers/isobars using multiple platforms and/or alternative technologies, such as currently evolving IM, (3) enhancing the ionization efficiency of especially neutral and low abundant species using the derivatization together with further improvement of the sensitivity of the analytical methods, (4) the absence of appropriate and reliable stable-isotope labelling methods or ISs for accurate quantitation, (5) the lack of commercially available reference materials that would enable standardization and reliable comparison among different laboratories, and (6) the lack of universal and fully automated software tools with unified annotation. Although addressing these issues is the subject of a large amount of ongoing research, fully-fledged high-throughput GSLs profiling in biological samples is still challenging rather than feasible. Consequently, significant efforts to overcome the aforementioned shortcomings should continue, as the translation of lipidomics into clinical practice is undoubtedly still an unresolved issue, and our understanding of GSL biology remains limited.

CRediT authorship contribution statement

Karel Hořejší: Writing – original draft, Visualization, Conceptualization. **Denisa Kolářová:** Writing – review & editing, Visualization. **Robert Jirásko:** Writing – review & editing, Supervision. **Michal Holčapek:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no conflicts of interest to this work.

Data availability

No data was used for the research described in the article.

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References

[1] Z. Guo, The structural diversity of natural glycosphingolipids (GSLs), J. Carbohydr. Chem. 41 (2022) 63–154.
[2] G. D'Angelo, T. Uemura, C.C. Chuang, E. Polishchuk, M. Santoro, H. Ohvo-Rekilä, T. Sato, G. Di Tullio, A. Varriale, S. D'Auria, et al., Vesicular and non-vesicular transport feed distinct glycosylation pathways in the golgi, Nature 501 (2013) 116–120, https://doi.org/10.1038/nature12423.
[3] H.J.F. MacCioni, R. Quiroga, M.L. Ferrari, Cellular and molecular biology of glycosphingolipid glycosylation, J. Neurochem. 117 (2011) 589–602, https://doi.org/10.1111/j.1471-4159.2011.07232.x.
[4] R.L. Schnaar, R. Sandhoff, M. Tiemeyer, T. Kinoshita, Glycosphingolipids, in: A. Varki, R.D. Cummings, J.D. Esko, P. Stanley (Eds.), Essentials of Glycobiology, Cold Spring Harbor Laboratory Press, New York, USA, 2022, p. 859. ISBN 978-1-621824-21-3.
[5] M.A. Chester, IUPAC-IUB joint commission on biochemical nomenclature (JCBN). Nomenclature of glycolipids–recommendations 1997, Eur. J. Biochem. 257 (1998) 293–298, https://doi.org/10.1046/j.1432-1327.1998.2570293.x.
[6] E. Fahy, S. Subramaniam, R.C. Murphy, M. Nishijima, C.R.H. Raetz, T. Shimizu, F. Spener, G. Van Meer, M.J.O. Wakelam, E.A. Dennis, Update of the LIPID MAPS comprehensive classification system for lipids, J. Lipid Res. 50 (2009) 9–14, https://doi.org/10.1194/jlr.R800095-JLR200.
[7] G. Liebisch, E. Fahy, J. Aoki, E.A. Dennis, T. Durand, C.S. Ejsing, M. Fedorova, I. Feussner, W.J. Griffiths, H. Köfeler, et al., Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures, J. Lipid Res. 61 (2020) 1539–1555, https://doi.org/10.1194/jlr.S120001025.
[8] S. Neelamegham, K. Aoki-Kinoshita, E. Bolton, M. Frank, F. Lisacek, T. Lütteke, N. O'Boyle, N.H. Packer, P. Stanley, P. Toukach, et al., Updates to the symbol nomenclature for glycans guidelines, Glycobiology 29 (2019) 620–624, https://doi.org/10.1093/glycob/cwz045.
[9] Y.A. Hannun, L.M. Obeid, Sphingolipids and their metabolism in physiology and disease, Nat. Rev. Mol. Cell Biol. 19 (2018) 175–191, https://doi.org/10.1038/nrm.2017.107.
[10] C.A. Lingwood, Glycosphingolipid functions, Cold Spring Harbor Perspect. Biol. 3 (2011) 1–26, https://doi.org/10.1101/cshperspect.a004788.
[11] T. Wennekes, R.J.B.H.N. Van Den Berg, R.G. Boot, G.A. Van Der Marel, H. S. Overkleeft, J.M.F.G. Aerts, Glycosphingolipids - nature, function, and pharmacological modulation, Angew. Chem. Int. Ed. 48 (2009) 8848–8869, https://doi.org/10.1002/anie.200902620.
[12] H. Schulze, K. Sandhoff, Lysosomal lipid storage diseases, Cold Spring Harbor Perspect. Biol. 3 (2011) 1–19, https://doi.org/10.1101/cshperspect.a004804.
[13] H.J. Willison, N. Yuki, Peripheral neuropathies and anti-glycolipid antibodies, Brain 125 (2002) 2591–2625, https://doi.org/10.1093/brain/awf272.
[14] P.J. Meikle, G. Wong, C.K. Barlow, B.A. Kingwell, Lipidomics: potential role in risk prediction and therapeutic monitoring for diabetes and cardiovascular disease, Pharmacol. Ther. 143 (2014) 12–23, https://doi.org/10.1016/j.pharmthera.2014.02.001.
[15] Y. Mohamed Abd-El-Halim, A. El Kaoutari, F. Silvy, M. Rubis, M. Bigonnet, J. Roques, J. Cros, R. Nicolle, J. Iovanna, N. Dusetti, et al., A glycosyltransferase gene signature to detect pancreatic ductal adenocarcinoma patients with poor prognosis, EBioMedicine 71 (2021) 103541, https://doi.org/10.1016/j.ebiom.2021.103541.
[16] S. Pietrobono, B. Stecca, Aberrant sialylation in cancer: biomarker and potential target for therapeutic intervention? Cancers 13 (2021) 2014, https://doi.org/10.3390/cancers13092014.
[17] D. Russo, L. Capolupo, J.S. Loomba, L. Sticco, G. D'Angelo, Glycosphingolipid metabolism in cell fate specification, J. Cell Sci. 131 (2018).
[18] L.M. Butler, Y. Perone, J. Dehairs, L.E. Lupien, V. de Laat, A. Talebi, M. Loda, W. B. Kinlaw, J.V. Swinnen, Lipids and cancer: emerging roles in pathogenesis, diagnosis and therapeutic intervention, Adv. Drug Deliv. Rev. 159 (2020) 245–293.
[19] D. Wolrab, R. Jirásko, M. Chocholoušková, O. Peterka, M. Holčapek, Oncolipidomics: mass spectrometric quantitation of lipids in cancer research, TrAC, Trends Anal. Chem. 120 (2019), https://doi.org/10.1016/j.trac.2019.04.012.
[20] H.F. Avela, H. Sirén, Advances in analytical tools and current statistical methods used in ultra-high-performance liquid chromatography-mass spectrometry of glycero-, glycerophospho- and sphingolipids, Int. J. Mass Spectrom. 457 (2020), https://doi.org/10.1016/j.ijms.2020.116408.
[21] A.C. Jirmo, C. Rossdam, R. Grychtol, C. Happle, R. Gerardy-Schahn, F.F. R. Buettner, G. Hansen, Differential expression patterns of glycosphingolipids and C-type lectin receptors on immune cells in absence of functional regulatory T cells, Immun Inflamm Dis 8 (2020) 512–522, https://doi.org/10.1002/iid3.334.
[22] C. Rossdam, S.A. Konze, A. Oberbeck, E. Rapp, R. Gerardy-Schahn, M. Von Itzstein, F.F.R. Buettner, Approach for profiling of glycosphingolipid glycosylation by multiplexed capillary gel electrophoresis coupled to laser-induced fluorescence detection to identify cell-surface markers of human pluripotent stem cells and derived cardiomyocytes, Anal. Chem. 91 (2019) 6413–6418, https://doi.org/10.1021/acs.analchem.9b01114.
[23] T. Züllig, M. Trötzmüller, H.C. Köfeler, Lipidomics from sample preparation to data analysis: a primer, Anal. Bioanal. Chem. 412 (2020) 2191–2209, https://doi.org/10.1007/s00216-019-02241-y.
[24] T. Züllig, H.C. Köfeler, High resolution mass spectrometry in lipidomics, Mass Spectrom. Rev. 40 (2021) 162–176, https://doi.org/10.1002/mas.21627.
[25] D. Wolrab, M. Chocholoušková, R. Jirásko, O. Peterka, M. Holčapek, Validation of lipidomic analysis of human plasma and serum by supercritical fluid chromatography–mass spectrometry and hydrophilic interaction liquid chromatography–mass spectrometry, Anal. Bioanal. Chem. 412 (2020) 2375–2388, https://doi.org/10.1007/s00216-020-02473-3.
[26] L. Yang, H. Nie, F. Zhao, S. Song, Y. Meng, Y. Bai, H. Liu, A novel online two-dimensional supercritical fluid chromatography/reversed phase liquid chromatography–mass spectrometry method for lipid profiling, Anal. Bioanal. Chem. 412 (2020) 2225–2235, https://doi.org/10.1007/s00216-019-02242-x.

[27] S.M. Camunas-Alberca, M. Moran-Garrido, J. Sáiz, A. Gil-de-la-Fuente, C. Barbas, A. Gradillas, Integrating the potential of ion mobility spectrometry-mass spectrometry in the separation and structural characterisation of lipid isomers, Front. Mol. Biosci. 10 (2023) 1–21, https://doi.org/10.3389/fmolb.2023.1112521.
[28] J.N. Dodds, E.S. Baker, Ion mobility spectrometry: fundamental concepts, instrumentation, applications, and the road ahead, J. Am. Soc. Mass Spectrom. 30 (2019) 2185–2195, https://doi.org/10.1007/s13361-019-02288-2.
[29] R. Wojcik, I.K. Webb, L. Deng, S.V.B. Garimella, S.A. Prost, Y.M. Ibrahim, E. S. Baker, R.D. Smith, Lipid and glycolipid isomer analyses using ultra-high resolution ion mobility spectrometry separations, Int. J. Mol. Sci. 18 (2017) 1–12, https://doi.org/10.3390/ijms18010183.
[30] J.C. May, R. Knochenmuss, J.C. Fjeldsted, J.A. McLean, Resolution of isomeric mixtures in ion mobility using a combined demultiplexing and peak deconvolution technique, Anal. Chem. 92 (2020) 9482–9492, https://doi.org/10.1021/acs.analchem.9b05718.
[31] K.V. Djambazova, M. Dufresne, L.G. Migas, A.R.S. Kruse, R. Van de Plas, R. M. Caprioli, J.M. Spraggins, MALDI TIMS IMS of disialoganglioside Isomers—GD1a and GD1b in murine brain tissue, Anal. Chem. 95 (2023) 1176–1183, https://doi.org/10.1021/acs.analchem.2c03939.
[32] M. Moran-Garrido, S.M. Camunas-Alberca, A. Gil-de-la Fuente, A. Mariscal, A. Gradillas, C. Barbas, J. Sáiz, Recent developments in data acquisition, treatment and analysis with ion mobility-mass spectrometry for lipidomics, Proteomics 22 (2022), https://doi.org/10.1002/pmic.202100328.
[33] J.C. May, K.L. Leaptrot, B.S. Rose, K.L.W. Moser, L. Deng, L. Maxon, D. Debord, J. A. McLean, Resolving power and collision cross section measurement accuracy of a prototype high-resolution ion mobility platform incorporating structures for lossless ion manipulation, J. Am. Soc. Mass Spectrom. 32 (2021) 1126–1137, https://doi.org/10.1021/jasms.1c00056.
[34] J. Li, T. Vosegaard, Z. Guo, Applications of nuclear magnetic resonance in lipid analyses: an emerging powerful tool for lipidomics studies, Prog. Lipid Res. 68 (2017) 37–56, https://doi.org/10.1016/j.plipres.2017.09.003.
[35] Z. Garádi, A. Tóth, T. Gáti, A. Dancsó, S. Béni, Utilizing the 1H-15N NMR methods for the characterization of isomeric human milk oligosaccharides, Int. J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms24032180.
[36] T. Hyötyläinen, M. Orešič, Bioanalytical techniques in nontargeted clinical lipidomics, Bioanalysis 8 (2016) 351–364.
[37] V.L. Stevens, E. Hoover, Y. Wang, K.A. Zanetti, Pre-analytical factors that affect metabolite stability in human urine, plasma, and serum: a review, Metabolites 9 (2019), https://doi.org/10.3390/metabo9080156.
[38] K. Jurowski, K. Kochan, J. Walczak, M. Barańska, W. Piekoszewski, B. Buszewski, Comprehensive review of trends and analytical strategies applied for biological samples preparation and storage in modern medical lipidomics: state of the art, TrAC, Trends Anal. Chem. 86 (2017) 276–289, https://doi.org/10.1016/j.trac.2016.10.014.
[39] G.B. Reis, J.C. Rees, A.A. Ivanova, Z. Kuklenyik, N.M. Drew, J.L. Pirkle, J.R. Barr, Stability of lipids in plasma and serum: effects of temperature-related storage conditions on the human lipidome, J. Mass Spectrometry Adv. Clin. Lab 22 (2021) 34–42, https://doi.org/10.1016/j.jmsacl.2021.10.002.
[40] D. Wolrab, M. Chocholoušková, R. Jirásko, O. Peterka, V. Mužáková, H. Študentová, B. Melichar, M. Holčapek, Determination of one year stability of lipid plasma profile and comparison of blood collection tubes using UHPSFC/MS and HILIC-UHPLC/MS, Anal. Chim. Acta 1137 (2020) 74–84, https://doi.org/10.1016/j.aca.2020.08.061.
[41] C.Z. Ulmer, J.P. Koelmel, C.M. Jones, T.J. Garrett, J.J. Aristizabal-Henao, H. W. Vesper, J.A. Bowden, A review of efforts to improve lipid stability during sample preparation and standardization efforts to ensure accuracy in the reporting of lipid measurements, Lipids 56 (2021) 3–16, https://doi.org/10.1002/lipd.12263.
[42] S.M. Hammad, J.S. Pierce, F. Soodavar, K.J. Smith, M.M. Al Gadban, B. Rembiesa, R.L. Klein, Y.A. Hannun, J. Bielawski, A. Bielawska, Blood sphingolipidomics in healthy humans: impact of sample collection methodology, J. Lipid Res. 51 (2010) 3074–3087, https://doi.org/10.1194/jlr.D008532.
[43] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509, https://doi.org/10.1016/s0021-9258(18)64849-5.
[44] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.
[45] V. Matyash, G. Liebisch, T.V. Kurzchalia, A. Shevchenko, D. Schwudke, Lipid extraction by methyl-terf-butyl ether for high-throughput lipidomics, J. Lipid Res. 49 (2008) 1137–1146, https://doi.org/10.1194/jlr.D700041-JLR200.
[46] L. Löfgren, G.B. Forsberg, M. Ståhlman, The BUME method: a new rapid and simple chloroform-free method for total lipid extraction of animal tissue, Sci. Rep. 6 (2016) 1–11, https://doi.org/10.1038/srep27688.
[47] Z.H. Alshehry, C.K. Barlow, J.M. Weir, Y. Zhou, M.J. McConville, P.J. Meikle, An efficient single phase method for the extraction of plasma lipids, Metabolites 5 (2015) 389–403, https://doi.org/10.3390/metabo5020389.
[48] G. Vale, S.A. Martin, M.A. Mitsche, B.M. Thompson, K.M. Eckert, J.G. McDonald, Three-phase liquid extraction: a simple and fast method for lipidomic workflows, J. Lipid Res. 60 (2019) 694–706, https://doi.org/10.1194/jlr.D090795.
[49] M. Höring, C. Stieglmeier, K. Schnabel, T. Hallmark, K. Ekroos, R. Burkhardt, G. Liebisch, Benchmarking one-phase lipid extractions for plasma lipidomics, Anal. Chem. 94 (2022) 12292–12296, https://doi.org/10.1021/acs.analchem.2c02117.
[50] C.C. Teo, W.P.K. Chong, E. Tan, N.B. Basri, Z.J. Low, Y.S. Ho, Advances in sample preparation and analytical techniques for lipidomics study of clinical samples, TrAC, Trends Anal. Chem. 66 (2015) 1–18, https://doi.org/10.1016/j.trac.2014.10.010.
[51] S. Albrecht, S. Vainauskas, H. Stöckmann, C. McManus, C.H. Taron, P.M. Rudd, Comprehensive profiling of glycosphingolipid glycans using a novel broad specificity endoglycoceramidase in a high-throughput workflow, Anal. Chem. 88 (2016) 4795–4802, https://doi.org/10.1021/acs.analchem.6b00259.
[52] C. Jin, S. Teneberg, Characterization of novel nonacid glycosphingolipids as biomarkers of human gastric adenocarcinoma, J. Biol. Chem. 298 (2022), https://doi.org/10.1016/j.jbc.2022.101732.
[53] K. Hořejší, R. Jirásko, M. Chocholoušková, D. Wolrab, D. Kahoun, M. Holčapek, Comprehensive identification of glycosphingolipids in human plasma using hydrophilic interaction liquid chromatography—electrospray ionization mass spectrometry, Metabolites 11 (2021) 1–24, https://doi.org/10.3390/metabo11030140.
[54] K. Hořejší, C. Jin, Z. Vaňková, R. Jirásko, O. Strouhal, B. Melichar, S. Teneberg, M. Holčapek, Comprehensive characterization of complex glycosphingolipids in human pancreatic cancer tissues, J. Biol. Chem. 299 (2023) 1–22, https://doi.org/10.1016/j.jbc.2023.102923.
[55] R.C. Barrientos, Q. Zhang, Recent advances in the mass spectrometric analysis of glycosphingolipidome - a review, Anal. Chim. Acta 1132 (2020) 134–155, https://doi.org/10.1016/j.aca.2020.05.051.Recent.
[56] K.-A. Karlsson, Preparation of total nonacid glycolipids for overlay analysis of receptors for bacteria and viruses and for other studies, Methods Enzymol. 138 (1987) 212–220, https://doi.org/10.1016/0076-6879(87)38018-8.
[57] M. Arends, M. Weber, C. Papan, M. Damm, M.A. Surma, C. Spiegel, M. Djannatian, S. Li, L. Connell, L. Johannes, et al., Ganglioside lipidomics of CNS myelination using direct infusion shotgun mass spectrometry, iScience 25 (2022), https://doi.org/10.1016/j.isci.2022.105323.
[58] R.C. Barrientos, Q. Zhang, Recent advances in the mass spectrometric analysis of glycosphingolipidome - a review, Anal. Chim. Acta 1132 (2020) 134–155, https://doi.org/10.1016/j.aca.2020.05.051.Recent.
[59] R.K. Saini, P. Prasad, X. Shang, Y.S. Keum, Advances in lipid extraction methods—a review, Int. J. Mol. Sci. 22 (2021) 1–19, https://doi.org/10.3390/ijms222413643.
[60] A. Barone, J. Benktander, C. Whiddon, C. Jin, C. Galli, S. Teneberg, M.E. Breimer, Glycosphingolipids of porcine, bovine, and equine pericardia as potential immune targets in bioprosthetic heart valve grafts, Xenotransplantation 25 (2018) 1–15, https://doi.org/10.1111/xen.12406.
[61] Z. Song, C. Duan, M. Shi, S. Li, Y. Guan, One-step preparation of ZrO2/SiO2 microspheres and modification with D-fructose 1,6-bisphosphate as stationary phase for hydrophilic interaction chromatography, J. Chromatogr. A 1522 (2017) 30–37, https://doi.org/10.1016/j.chroma.2017.09.046.
[62] S. Khoury, E. Masson, E. Sibille, S. Cabaret, O. Berdeaux, Rapid sample preparation for ganglioside analysis by liquid chromatography mass spectrometry, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1137 (2020) 121956, https://doi.org/10.1016/j.jchromb.2019.121956.
[63] H. Nagasawa, S. Miyazaki, M. Kyogashima, Simple separation of glycosphingolipids in the lower phase of a folch's partition from crude lipid fractions using zirconium dioxide, Glycoconj. J. 39 (2022) 789–795, https://doi.org/10.1007/s10719-022-10080-w.
[64] R. Jin, L. Li, J. Feng, Z. Dai, Y.W. Huang, Q. Shen, Zwitterionic hydrophilic interaction solid-phase extraction and multi-dimensional mass spectrometry for shotgun lipidomic study of Hypophthalmichthys nobilis, Food Chem. 216 (2017) 347–354, https://doi.org/10.1016/j.foodchem.2016.08.074.
[65] R. Hájek, M. Lísa, M. Khalikova, R. Jirásko, E. Cífková, V. Študent, D. Vrána, L. Opálka, K. Vávrová, M. Matzenauer, et al., HILIC/ESI-MS determination of gangliosides and other polar lipid classes in renal cell carcinoma and surrounding normal tissues, Anal. Bioanal. Chem. 410 (2018) 6585–6594, https://doi.org/10.1007/s00216-018-1263-8.
[66] R. Wojcik, I.K. Webb, L. Deng, S.V.B. Garimella, S.A. Prost, Y.M. Ibrahim, E. S. Baker, R.D. Smith, Lipid and glycolipid isomer analyses using ultra-high resolution ion mobility spectrometry separations, Int. J. Mol. Sci. 18 (2017) 1–12, https://doi.org/10.3390/ijms18010183.
[67] A.H. Merrill, M.C. Sullards, Opinion article on lipidomics: inherent challenges of lipidomic analysis of sphingolipids, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862 (2017) 774–776, https://doi.org/10.1016/j.bbalip.2017.01.009.
[68] Y. Bin Han, L.Q. Chen, Z. Li, Y.M. Tan, Y. Feng, G.Y. Yang, Structural insights into the broad substrate specificity of a novel endoglycoceramidase i belonging to a new subfamily of GH5 glycosidases, J. Biol. Chem. 292 (2017) 4789–4800, https://doi.org/10.1074/jbc.M116.763821.
[69] M. Holčapek, M. Lísa, Silver-ion liquid chromatography - mass spectrometry, in: M. Holčapek, W.C. Byrdwell (Eds.), Handbook of Advanced Chromatography/Mass Spectrometry Techniques, Elsevier, London, UK, 2017, p. 520. ISBN 9780128117323.
[70] M. Lísa, M. Holčapek, Characterization of triacylglycerol enantiomers using chiral HPLC/APCI-MS and synthesis of enantiomeric triacylglycerols, Anal. Chem. 85 (2013) 1852–1859, https://doi.org/10.1021/ac303237a.
[71] Z. Vaňková, O. Peterka, M. Chocholoušková, D. Wolrab, R. Jirásko, M. Holčapek, Retention dependences support highly confident identification of lipid species in human plasma by reversed-phase UHPLC/MS, Anal. Bioanal. Chem. 414 (2022) 319–331, https://doi.org/10.1007/s00216-021-03492-4.
[72] D. Wang, K. Madunić, T. Zhang, O.A. Mayboroda, G.S.M. Lageveen-Kammeijer, M. Wuhrer, High diversity of glycosphingolipid glycans of colorectal cancer cell lines reflects the cellular differentiation phenotype, Mol. Cell. Proteomics 21 (2022) 1–14, https://doi.org/10.1016/j.mcpro.2022.100239.

[73] M. Mank, P. Welsch, A.J.R. Heck, B. Stahl, Label-free targeted LC-ESI-MS2 analysis of human milk oligosaccharides (HMOS) and related human milk groups with enhanced structural selectivity, Anal. Bioanal. Chem. 411 (2019) 231–250, https://doi.org/10.1007/s00216-018-1434-7.
[74] B.G. Cho, W. Peng, Y. Mechref, Separation of permethylated O-glycans, free oligosaccharides, and glycosphingolipid-glycans using porous graphitized carbon (Pgc) column, Metabolites 10 (2020) 1–12, https://doi.org/10.3390/metabo10110433.
[75] J.C. May, R. Knochenmuss, J.C. Fjeldsted, J.A. McLean, Resolution of isomeric mixtures in ion mobility using a combined demultiplexing and peak deconvolution technique, Anal. Chem. 92 (2020) 9482–9492, https://doi.org/10.1021/acs.analchem.9b05718.
[76] H. Xu, F.R. Boucher, T.T. Nguyen, G.P. Taylor, J.J. Tomlinson, R.A. Ortega, B. Simons, M.G. Schlossmacher, R. Saunders-Pullman, W. Shaw, et al., DMS as an orthogonal separation to LC/ESI/MS/MS for quantifying isomeric cerebrosides in plasma and cerebrospinal fluid, J. Lipid Res. 60 (2019) 200–211, https://doi.org/10.1194/jlr.D089797.
[77] A. Bednařík, S. Bölsker, J. Soltwisch, K. Dreisewerd, An on-tissue Paternò–Büchi reaction for Localization of carbon–carbon double bonds in phospholipids and glycolipids by matrix-assisted laser-desorption–ionization mass-spectrometry imaging, Angew. Chem. Int. Ed. 57 (2018) 12092–12096, https://doi.org/10.1002/anie.201806635.
[78] R.C. Barrientos, Q. Zhang, Fragmentation behavior and gas-phase structures of cationized glycosphingolipids in ozone-induced dissociation mass spectrometry, J. Am. Soc. Mass Spectrom. 30 (2019) 1609–1620, https://doi.org/10.1007/s13361-019-02267-7.
[79] H. Zhang, M. Xu, X. Shi, Y. Liu, Z. Li, J.C. Jagodinsky, M. Ma, N.V. Welham, Z. S. Morris, L. Li, Quantification and molecular imaging of fatty acid isomers from complex biological samples by mass spectrometry, Chem. Sci. 12 (2021) 8115–8122, https://doi.org/10.1039/d1sc01614h.
[80] J. Zhang, Z. Zhang, T. Jiang, Z. Zhang, W. Zhang, W. Xu, Rapidly identifying and quantifying of unsaturated lipids with carbon-carbon double bond isomers by photoepoxidation, Talanta 260 (2023) 124575, https://doi.org/10.1016/j.talanta.2023.124575.
[81] S. Tang, H. Cheng, X. Yan, On-demand electrochemical epoxidation in nano-electrospray ionization mass spectrometry to locate carbon–carbon double bonds, Angew. Chem. Int. Ed. 59 (2020) 209–214, https://doi.org/10.1002/anie.201911070.
[82] K. Luo, H. Chen, R.N. Zare, Location of carbon-carbon double bonds in unsaturated lipids using microdroplet mass spectrometry, Analyst 146 (2021) 2550–2558, https://doi.org/10.1039/d0an02396e.
[83] Y. Zhao, H. Zhao, X. Zhao, J. Jia, Q. Ma, S. Zhang, X. Zhang, H. Chiba, S.P. Hui, X. Ma, Identification and quantitation of C=C location isomers of unsaturated fatty acids by epoxidation reaction and tandem mass spectrometry, Anal. Chem. 89 (2017) 10270–10278, https://doi.org/10.1021/acs.analchem.7b01870.
[84] D. Unsihuay, P. Su, H. Hu, J. Qiu, S. Kuang, Y. Li, X. Sun, S.K. Dey, J. Laskin, Imaging and analysis of isomeric unsaturated lipids through online photochemical derivatization of carbon–carbon double bonds, Angew. Chem. Int. Ed. 60 (2021) 7559–7563, https://doi.org/10.1002/anie.202016734.
[85] H. Lu, H. Zhang, S. Xu, L. Li, Review of recent advances in lipid analysis of biological samples via ambient ionization mass spectrometry, Metabolites 11 (2021) 781, https://doi.org/10.3390/metabo11110781.
[86] F. Xia, J.B. Wan, Chemical derivatization strategy for mass spectrometry-based lipidomics, Mass Spectrom. Rev. 42 (2023) 432–452, https://doi.org/10.1002/mas.21729.
[87] W. Zhang, R. Jian, J. Zhao, Y. Liu, Y. Xia, Deep-lipidotyping by mass spectrometry: recent technical advances and applications, J. Lipid Res. 63 (2022) 100219, https://doi.org/10.1016/j.jlr.2022.100219.
[88] J. Zhang, X. Huo, C. Guo, X. Ma, H. Huang, J. He, X. Wang, F. Tang, Rapid imaging of unsaturated lipids at an isomeric level achieved by controllable oxidation, Anal. Chem. 93 (2021) 2114–2124, https://doi.org/10.1021/acs.analchem.0c03888.
[89] K. Aoki, A.D. Heaps, K.A. Strauss, M. Tiemeyer, Mass spectrometric quantification of plasma glycosphingolipids in human GM3 ganglioside deficiency, Clin.l Mass Spectrometry 14 (2019) 106–114, https://doi.org/10.1016/j.clinms.2019.03.001.
[90] R.C. Barrientos, Q. Zhang, Differential isotope labeling by permethylation and reversed-phase liquid chromatography-mass spectrometry for relative quantification of intact neutral glycolipids in mammalian cells, Anal. Chem. 91 (2019) 9673–9681, https://doi.org/10.1021/acs.analchem.9b01206.
[91] D.F. Smith, P.A. Prieto, Special considerations for glycolipids and their purification, Curr. Protoc. Mol. Biol. 22 (1993) 1–13, https://doi.org/10.1002/0471142727.mb1703s22.
[92] S. Sekiya, Y. Wada, K. Tanaka, Derivatization for stabilizing sialic acids in MALDI-MS study , we tried to stabilize the moiety by amidation , as in, Society 77 (2005) 4962–4968.
[93] Y. Liu, L. Yang, H. Li, J. Liu, R. Tian, Derivatization strategy for sensitive identification of neutral and acidic glycosphingolipids using RPLC-MS, Int. J. Mass Spectrom. 482 (2022) 116937, https://doi.org/10.1016/j.ijms.2022.116937.
[94] H. Hanamatsu, T. Nishikaze, N. Miura, J. Piao, K. Okada, S. Sekiya, S. Iwamoto, N. Sakamoto, K. Tanaka, J.I. Furukawa, Sialic acid linkage specific derivatization of glycosphingolipid glycans by ring-opening aminolysis of lactones, Anal. Chem. 90 (2018) 13193–13199, https://doi.org/10.1021/acs.analchem.8b02775.
[95] R.C. Barrientos, Q. Zhang, Isobaric labeling of intact gangliosides toward multiplexed LC-MS/MS-Based quantitative analysis, Anal. Chem. 90 (2018) 2578–2586, https://doi.org/10.1021/acs.analchem.7b04044.
[96] O. Peterka, R. Jirásko, Z. Vaňková, M. Chocholoušková, D. Wolrab, J. Kulhánek, F. Bureš, M. Holčapek, Simple and reproducible derivatization with benzoyl chloride: improvement of sensitivity for multiple lipid classes in RP-UHPLC/MS, Anal. Chem. 93 (2021) 13835–13843, https://doi.org/10.1021/acs.analchem.1c02463.
[97] M. Wang, C. Wang, X. Han, Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry—what, how and why? Mass Spectrom. Rev. 36 (2017) 693–714, https://doi.org/10.1002/mas.21492.
[98] K.A. Lippa, J.J. Aristizabal-Henao, R.D. Beger, J.A. Bowden, C. Broeckling, C. Beecher, W. Clay Davis, W.B. Dunn, R. Flores, R. Goodacre, et al., Reference materials for MS-based untargeted metabolomics and lipidomics: a review by the metabolomics quality assurance and quality control consortium (MQACC), Metabolomics 18 (2022) 1–29, https://doi.org/10.1007/s11306-021-01848-6.
[99] M. Lange, M. Fedorova, Evaluation of lipid quantification accuracy using HILIC and RPLC MS on the example of NIST® SRM® 1950 metabolites in human plasma, Anal. Bioanal. Chem. 412 (2020) 3573–3584, https://doi.org/10.1007/s00216-020-02576-x.
[100] M. Holčapek, G. Liebisch, K. Ekroos, Lipidomic analysis, Anal. Chem. 90 (2018) 4249–4257, https://doi.org/10.1021/acs.analchem.7b05395.
[101] M.A. Jaber, B. de Falco, S. Abdelrazig, C.A. Ortori, D.A. Barrett, D.H. Kim, Advantages of using biologically generated 13C-labelled multiple internal standards for stable isotope-assisted LC-MS-based lipidomics, Anal. Methods 15 (2023) 2925–2934, https://doi.org/10.1039/d3ay00460k.
[102] R.S. Rohokale, Q. Li, Z. Guo, A diversity-oriented strategy for chemical synthesis of glycosphingolipids: synthesis of glycosphingolipid LcGg4 and its analogues and derivatives, J. Org. Chem. 86 (2021) 1633–1648, https://doi.org/10.1021/acs.joc.0c02490.
[103] Y.C. Chiang, C.Y. Wu, P.Y. Chiang, A.K. Adak, C.C. Lin, A concise chemoenzymatic total synthesis of neutral globo-series glycosphingolipids globo A and globo B, and Forssman and para-Forssman Antigens, Glycoconj. J. 40 (2023) 551–563, https://doi.org/10.1007/s10719-023-10133-8.
[104] N.R. Reddy, Stable labeled isotopes as internal standards: a critical review, Modern appl. Pharmacy Pharmacol. 1 (2017) 1–4, https://doi.org/10.31031/mapp.2017.01.000508.
[105] E. Rampler, Y. El Abiead, H. Schoeny, M. Rusz, F. Hildebrand, V. Fitz, G. Koellensperger, Recurrent topics in mass spectrometry-based metabolomics and lipidomics - standardization, coverage, and throughput, Anal. Chem. 93 (2021) 519–545, https://doi.org/10.1021/acs.analchem.0c04698.
[106] A. Triebl, M.R. Wenk, Analytical considerations of stable isotope labelling in lipidomics, Biomolecules 8 (2018), https://doi.org/10.3390/biom8040151.
[107] A.F. Salvador, C.R. Shyu, E.J. Parks, Measurement of lipid flux to advance translational research: evolution of classic methods to the future of precision health, Exp. Mol. Med. 54 (2022) 1348–1353, https://doi.org/10.1038/s12276-022-00838-5.
[108] M. Chocholoušková, D. Wolrab, R. Jirásko, H. Študentová, B. Melichar, M. Holčapek, Intra-laboratory comparison of four analytical platforms for lipidomic quantitation using hydrophilic interaction liquid chromatography or supercritical fluid chromatography coupled to quadrupole - time-of-flight mass spectrometry, Talanta 231 (2021), https://doi.org/10.1016/j.talanta.2021.122367.
[109] J.A. Bowden, A. Heckert, C.Z. Ulmer, C.M. Jones, J.P. Koelmel, L. Abdullah, L. Ahonen, Y. Alnouti, A.M. Armando, J.M. Asara, et al., Harmonizing lipidomics: NIST interlaboratory comparison exercise for lipidomics using SRM 1950-metabolites in frozen human plasma, J. Lipid Res. 58 (2017) 2275–2288, https://doi.org/10.1194/jlr.M079012.
[110] A. Triebl, B. Burla, J. Selvalatchmanan, J. Oh, S.H. Tan, M.Y. Chan, N.A. Mellet, P. J. Meikle, F. Torta, M.R. Wenk, Shared reference materials harmonize lipidomics across MS-based detection platforms and laboratories, J. Lipid Res. 61 (2020) 105–115, https://doi.org/10.1194/jlr.D119000393.
[111] G. Olshansky, C. Giles, A. Salim, P.J. Meikle, Challenges and opportunities for prevention and removal of unwanted variation in lipidomic studies, Prog. Lipid Res. 87 (2022) 101177, https://doi.org/10.1016/j.plipres.2022.101177.
[112] H.C. Köfeler, R. Ahrends, E.S. Baker, K. Ekroos, X. Han, N. Hoffmann, M. Holcapek, M.R. Wenk, G. Liebisch, Recommendations for good practice in Ms-based lipidomics, J. Lipid Res. 62 (2021) 1–13, https://doi.org/10.1016/j.jlr.2021.100138.
[113] N.R. Zhang, N.G. Hatcher, K. Ekroos, K. Kedia, M. Kandebo, J.N. Marcus, S. M. Smith, K.P. Bateman, D.S. Spellman, Validation of a multiplexed and targeted lipidomics assay for accurate quantification of lipidomes, J. Lipid Res. 63 (2022) 100218, https://doi.org/10.1016/j.jlr.2022.100218.
[114] H.C. Köfeler, T.O. Eichmann, R. Ahrends, J.A. Bowden, N. Danne-Rasche, E. A. Dennis, M. Fedorova, W.J. Griffiths, X. Han, J. Hartler, et al., Quality control requirements for the correct annotation of lipidomics data, Nat. Commun. 12 (2021) 19–22, https://doi.org/10.1038/s41467-021-24984-y.
[115] M. Ghorasaini, Y. Mohammed, J. Adamski, L. Bettcher, J.A. Bowden, M. Cabruja, K. Contrepois, M. Ellenberger, B. Gajera, M. Haid, et al., Cross-laboratory standardization of preclinical lipidomics using differential mobility spectrometry and multiple reaction monitoring, Anal. Chem. 93 (2021) 16369–16378, https://doi.org/10.1021/acs.analchem.1c02826.
[116] J.E. Kyle, L. Aimo, A.J. Bridge, G. Clair, M. Fedorova, J.B. Helms, M.R. Molenaar, Z. Ni, M. Orešič, D. Slenter, et al., Interpreting the lipidome: bioinformatic

approaches to embrace the complexity, Metabolomics 17 (2021), https://doi.org/10.1007/s11306-021-01802-6.
[117] Z. Ni, M. Wölk, G. Jukes, K. Mendivelso Espinosa, R. Ahrends, L. Aimo, J. Alvarez-Jarreta, S. Andrews, R. Andrews, A. Bridge, et al., Guiding the choice of informatics software and tools for lipidomics research applications, Nat. Methods 20 (2023) 193–204, https://doi.org/10.1038/s41592-022-01710-0.
[118] W.J. Lin, P.C. Shen, H.C. Liu, Y.C. Cho, M.K. Hsu, I.C. Lin, F.H. Chen, J.C. Yang, W.L. Ma, W.C. Cheng, LipidSig: a web-based tool for lipidomic data analysis, Nucleic Acids Res. 49 (2021) W336–W345, https://doi.org/10.1093/nar/gkab419.
[119] A. Ceroni, K. Maass, H. Geyer, R. Geyer, A. Dell, S.M. Haslam, GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans, J. Proteome Res. 7 (2008) 1650–1659, https://doi.org/10.1021/pr7008252.
[120] S.B. Agravat, J.H. Saltz, R.D. Cummings, D.F. Smith, GlycoPattern: a web platform for glycan array mining, Bioinformatics 30 (2014) 3417–3418, https://doi.org/10.1093/bioinformatics/btu559.
[121] L. Gotz, J.L. Abrahams, J. Mariethoz, P.M. Rudd, N.G. Karlsson, N.H. Packer, M. P. Campbell, F. Lisacek, GlycoDigest: a tool for the targeted use of exoglycosidase digestions in glycan structure determination, Bioinformatics 30 (2014) 3131–3133, https://doi.org/10.1093/bioinformatics/btu425.
[122] N. Hoffmann, G. Mayer, C. Has, D. Kopczynski, F. Al Machot, D. Schwudke, R. Ahrends, K. Marcus, M. Eisenacher, M. Turewicz, A current encyclopedia of bioinformatics tools, data formats and resources for mass spectrometry lipidomics, Metabolites 12 (2022), https://doi.org/10.3390/metabo12070584.
[123] D.H. Ross, J. Guo, A. Bilbao, T. Huan, R.D. Smith, X. Zheng, Evaluating software tools for lipid identification from ion mobility spectrometry–mass spectrometry lipidomics data, Molecules 28 (2023) 1–13, https://doi.org/10.3390/molecules28083483.
[124] F. Yan, H. Zhao, Y. Zeng, Lipidomics: a promising cancer biomarker, Clin. Transl. Med. 7 (2018) 21–23, https://doi.org/10.1186/s40169-018-0199-0.
[125] X. Wang, Clinical trans-omics: an integration of clinical phenomes with molecular multiomics, Cell Biol. Toxicol. 34 (2018) 163–166, https://doi.org/10.1007/s10565-018-9431-3.
[126] M. Vogeser, A.K. Bendt, From research cohorts to the patient - a role for “omics” in diagnostics and laboratory medicine? Clin. Chem. Lab. Med. 61 (2023) 974–980, https://doi.org/10.1515/cclm-2022-1147.
[127] D. Wolrab, R. Jirásko, E. Cífková, M. Höring, D. Mei, M. Chocholoušková, O. Peterka, J. Idkowiak, T. Hrnčiarová, L. Kuchař, et al., Lipidomic profiling of human serum enables detection of pancreatic cancer, Nat. Commun. 13 (2022) 1–16, https://doi.org/10.1038/s41467-021-27765-9.
[128] T. Bien, M. Perl, A.C. MacHmüller, U. Nitsche, A. Conrad, L. Johannes, J. Müthing, J. Soltwisch, K.P. Janssen, K. Dreisewerd, MALDI-2 mass spectrometry and immunohistochemistry imaging of Gb3Cer, Gb4Cer, and further glycosphingolipids in human colorectal cancer tissue, Anal. Chem. 92 (2020) 7096–7105, https://doi.org/10.1021/acs.analchem.0c00480.
[129] M. Jeyakumar, T.D. Butters, R.A. Dwek, F.M. Platt, Glycosphingolipid lysosomal storage diseases: therapy and pathogenesis, Neuropathol. Appl. Neurobiol. 28 (2002) 343–357, https://doi.org/10.1046/j.1365-2990.2002.00422.x.
[130] P. Fredman, The role of antiglycolipid antibodies in neurological disorders, Ann. N. Y. Acad. Sci. 19 (1988) 341–352, https://doi.org/10.1111/j.1749-6632.1998.tb09686.x.
[131] N. Yuki, Infectious origins of, and molecular mimicry in, Guillain-Barré and Fisher syndromes, Lancet Infect. Dis. 1 (2001) 29–37, https://doi.org/10.1016/S1473-3099(01)00019-6.
[132] O. Vvedenskaya, M. Holčapek, M. Vogeser, K. Ekroos, P.J. Meikle, A.K. Bendt, Clinical lipidomics – a community-driven roadmap to translate research into clinical applications, J. Mass Spectrometry Adv. Clin. Lab 24 (2022) 1–4, https://doi.org/10.1016/j.jmsacl.2022.02.002.
[133] B. Burla, M. Arita, M. Arita, A.K. Bendt, A. Cazenave-Gassiot, E.A. Dennis, K. Ekroos, X. Han, K. Ikeda, G. Liebisch, et al., MS-based lipidomics of human blood plasma: a community-initiated position paper to develop accepted guidelines, J. Lipid Res. 59 (2018) 2001–2017, https://doi.org/10.1194/jlr.S087163.
[134] M.J. Ryan, A. Grant-St James, N.G. Lawler, M.W. Fear, E. Raby, F.M. Wood, G. L. Maker, J. Wist, E. Holmes, J.K. Nicholson, et al., Comprehensive lipidomic workflow for multicohort population phenotyping using stable isotope dilution targeted liquid chromatography-mass spectrometry, J. Proteome Res. 22 (2023) 1419–1433, https://doi.org/10.1021/acs.jproteome.2c00682.
[135] D. Wolrab, R. Jirásko, O. Peterka, J. Idkowiak, M. Chocholoušková, Z. Vaňková, K. Hořejší, I. Brabcová, D. Vrána, H. Študentová, et al., Plasma lipidomic profiles of kidney, breast and prostate cancer patients differ from healthy controls, Sci. Rep. 11 (2021) 1–14, https://doi.org/10.1038/s41598-021-99586-1.
[136] R. Saito, K. Yoshimura, K. Shoda, S. Furuya, H. Akaike, Y. Kawaguchi, T. Murata, K. Ogata, T. Iwano, S. Takeda, et al., Diagnostic significance of plasma lipid markers and machine learning-based algorithm for gastric cancer, Oncol. Lett. 21 (2021) 1–8, https://doi.org/10.3892/ol.2021.12666.
[137] C. Yang, S. Zhou, J. Zhu, H. Sheng, W. Mao, Z. Fu, Z. Chen, Plasma lipid-based machine learning models provides a potential diagnostic tool for colorectal cancer patients, Clin. Chim. Acta 536 (2022) 191–199, https://doi.org/10.1016/j.cca.2022.09.002.
[138] T. Brito-Rocha, V. Constâncio, R. Henrique, C. Jerónimo, Shifting the cancer screening paradigm: the rising potential of blood-based multi-cancer early detection tests, Cells 12 (2023), https://doi.org/10.3390/cells12060935.
[139] H.B. Ferreira, I.M.S. Guerra, T. Melo, H. Rocha, A.S.P. Moreira, A. Paiva, M. R. Domingues, Dried blood spots in clinical lipidomics: optimization and recent findings, Anal. Bioanal. Chem. (2022) 7085–7101, https://doi.org/10.1007/s00216-022-04221-1.
[140] M.U. Thangavelu, B. Wouters, A. Kindt, I.K.M. Reiss, T. Hankemeier, Blood microsampling technologies: innovations and applications in 2022, Anal. Sci. Adv 4 (2023) 154–180.
[141] V.B. O’Donnell, K. Ekroos, G. Liebisch, M. Lipidomics Wakelam, Current state of the art in a fast moving field, Wiley Interdiscip Rev Syst Biol Med 12 (2019) 1–6, https://doi.org/10.1002/wsbm.1466.
[142] T.G. Meikle, K. Huynh, C. Giles, P.J. Meikle, Clinical lipidomics: realizing the potential of lipid profiling, J. Lipid Res. 63 (2021) 100127, https://doi.org/10.1016/J.JLR.2021.100127.
[143] T.H. Kuo, H.H. Chung, H.Y. Chang, C.W. Lin, M.Y. Wang, T.L. Shen, C.C. Hsu, Deep lipidomics and molecular imaging of unsaturated lipid isomers: a universal strategy initiated by MCPBA epoxidation, Anal. Chem. 91 (2019) 11905–11915, https://doi.org/10.1021/acs.analchem.9b02667.