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Recent methodological developments in Data-Dependent Analysis and Data-Independent Analysis workflows for exhaustive lipidome coverage

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13 **Keywords: Lipidomics, Data-Dependent Acquisition, Data-Independent Acquisition, High-**
14 **Resolution Mass Spectrometry, Lipid annotation**

15 **Abstract**

16 Untargeted lipidomics applied to biological samples typically involves the coupling of separation
17 methods to high-resolution mass spectrometry (HRMS). Getting an exhaustive coverage of the
18 lipidome with a high confidence in structure identification is still highly challenging due to the wide
19 concentration range of lipids in complex matrices and the presence of numerous isobaric and isomeric
20 species. The development of innovative separation methods and HRMS(/MS) acquisition workflows
21 helped improving the situation but issues still remain regarding confident structure characterization.

22 To overcome these issues, thoroughly optimized MS/MS acquisition methods are needed. For this
23 purpose, different methodologies have been developed to enable MS and MS/MS acquisition in
24 parallel. Those methodologies, derived from the proteomics, are referred to Data Dependent
25 Acquisition (DDA) and Data Independent Acquisition (DIA).

26 In this context, this perspective paper presents the latest developments of DDA- and DIA-based
27 workflows for lipidomics and lists available bioinformatic tools for the analysis of resulting spectral
28 data.

29

30 1 Introduction

31 Lipids represent a large class of primary metabolites playing vital biological functions to maintain
32 homeostasis in living organisms, serving as energy storage and cell signaling processes (Züllig et al.,
33 2020). They are also involved in a large number of heterogeneous diseases including cancer,
34 neurodegenerative and metabolic disorders. Lipids are classified in 8 categories according to the Lipid
35 Maps classification system (Fahy et al., 2005, 2009; Liebisch et al., 2020) available online through the
36 curated Lipid MAPS Structure Database (LMSD) gathering almost 48,000 unique lipid species in 2022
37 (<https://www.lipidmaps.org/>). Those categories can be further separated into classes, in which lipids
38 share some building blocks but also differ by the length of alkyl chains and number of unsaturations
39 creating a high diversity of closely related lipid species making their distinction highly challenging
40 (Quehenberger et al., 2010; Harayama and Riezman, 2018).

41 Lipidomics can be defined as a system-level analysis of lipids and their interacting factors (Wenk,
42 2005). Lipidomics is a subset of metabolomics requiring dedicated sample preparation, as lipids are
43 mostly soluble in organic solvents (*e.g.* methyl tert-butyl ether, chloroform and methanol for Bligh-
44 Dyer and Folch extractions), as well as specific analytical tools to qualitatively and quantitatively
45 characterize complex samples.

46 One of the major challenges in lipidomics is to distinguish isobaric and isomeric lipid species by
47 implementing either optimized hyphenated mass spectrometry (MS)-based methods and/or tandem
48 mass spectrometry approaches. The fine structure elucidation of complex lipids, such as
49 glycerophospholipids, requires the identification of the polar head group, the chain lengths and the
50 position of the fatty acids together with the number and position of unsaturations or other chemical
51 modifications of the lipid backbone. The precise characterization of lipid species is mandatory to
52 interpret their biological or chemical roles in a defined context (Wenk, 2010).

53 Among the technical developments performed in the last 70 years, gas chromatography coupled to
54 electron ionization - mass spectrometry (GC-EI-MS) can be considered as the first major tools towards
55 lipidomics. Even if the techniques required complex sample preparation including subclasses
56 separation and chemical derivatization, GC-MS allowed efficient profiling of the fatty acids composing
57 complex lipids (Chiu and Kuo, 2020).

58 The multiple ionization sources and particularly soft ionization such as Electrospray Ionization (ESI),
59 Matrix-Assisted Laser Desorption/Ionization (MALDI) and Atmospheric Pressure Chemical/Photo-
60 Ionization (APCI/APPI), have made mass spectrometry as the technique of preference to perform
61 lipidome characterization and quantification. Also, Atmospheric Pressure ionization sources are mostly
62 employed for compounds difficult to ionize in ESI, mostly represented by neutral lipids such as
63 triacylglycerols or carotenoids (Byrdwell, 2001; Imbert et al., 2012).

64 The advent of liquid chromatography coupled to mass spectrometry (LC-MS) in the 80's offered new
65 perspectives as intact complex lipids can be directly separated, detected and isolated for tandem mass
66 spectrometry (MS/MS) experiments. Nevertheless, the low mass resolution instruments available at
67 that time did not offer sufficient high mass measurement accuracy and led to difficulty in distinguishing
68 isobaric species. The commercialization of fast and robust high-resolution (HR)/high-mass accuracy
69 tandem mass spectrometers paved the route to the analysis of high-mass lipids thanks to extended mass
70 capacities and dynamic ranges (up to 4 decades on modern instruments), high ionization rate for
71 increased sensitivity in both polarities and for all lipid sub-classes. Time-of-flight (TOF) mass
72 analyzers show the highest repetition rate. In terms of mass accuracy and resolving power, Fourier

73 Transform mass spectrometers, such as Orbitrap and Fourier-transform ion cyclotron resonance
74 (FTICR) instruments, are overpassing the TOF performances in spite of their slower acquisition rates
75 limiting the number of data points per chromatographic peak. The Orbitrap-based instruments, since
76 their first commercialization with the LTQ-Orbitrap in 2006, have witnessed innovations in sensitivity,
77 resolution and scan speed (Eliuk and Makarov, 2015). The lipidome investigation can be now achieved
78 at high throughput by either a shotgun approach (direct introduction into the mass spectrometer) or
79 using hyphenated MS-based techniques such as normal or reverse liquid phase or supercritical fluid
80 chromatography (Rustam and Reid, 2018). The common analysis workflow for those methods is based
81 on the acquisition at the MS level of robust data for biological and technical replicates together with
82 quality control (QC) injections to correct any deviation of the analytical chain. Then the identification
83 of relevant markers or compounds differentiating sample groups is further accomplished by tandem
84 mass spectrometry (MS/MS) following multiple injections into the LC-MS/MS system using targeted
85 MS/MS workflows. MS/MS spectral data are necessary for the elucidation structure of lipids. The
86 major part of the existing lipid databases mostly includes tandem mass spectra acquired under low-
87 energy non-resonant collision-induced dissociation (CID) or higher energy collision dissociation
88 (HCD) conditions on hybrid TOF and Orbitrap-based instruments. But, multiple types of ion activation
89 modes (*i.e.* resonant and non-resonant activation modes) exist and can be employed in MS/MS and are
90 currently used to a lesser extent for high throughput lipidomics workflows.

91 As for classical proteomics approaches, the parallel acquisition of HRMS and MS/MS data can be
92 achieved using either a Data-Dependent Analysis (DDA) or a Data-Independent Analysis (DIA)
93 approach. In the following Perspective article, technical and practical considerations on DDA and DIA
94 for lipidomics will be discussed to offer an overview of the latest developments and future trends in
95 the field of lipidomics.

96 **2 DDA-based acquisition workflows**

97 The DDA fragmentation method allows successive acquisitions of MS and MS/MS data. The most
98 widely used DDA acquisition method is often designated as the “DDA Top N”. It consists of a cycle
99 of a single HRMS scan followed by the automatic selection and further fragmentation of the N most
100 intense signals in a defined m/z range (Figure 1). The first HRMS scan offers the unique possibility of
101 getting high mass accuracy measurements, typically below 1-2 ppm. Unambiguous chemical formula
102 can be easily determined for small lipids, below m/z 300-400 but much higher mass accuracy
103 (< 0.1 ppm) would be required to determine the elemental composition of complex lipids.
104 Then automatic MS/MS scans are programmed based on rules such as peak intensity and mass range
105 on HRMS data. This method enables fragmenting a high number of ions and leads to high-quality and
106 high-purity MS/MS spectra since the selection window is restricted to monoisotopic ions.
107 Nevertheless, precursor ions selection is a (semi-)stochastic event suffering from low analytical
108 reproducibility and favoring selection of the most abundant (but sometimes biologically irrelevant)
109 precursor ions to the detriment of other co-eluting relevant ones (Fenaille et al., 2017). These issues
110 can be partially overcome through the implementation of exclusion rules, typical after one or several
111 consecutive MS/MS experiments, . The number of selected ions must be thoroughly optimized
112 considering the MS/MS overall duty cycle (usually few tens to hundreds of milliseconds per MS/MS
113 spectrum) and the LC peak width. A too high number of precursor ions will limit the number of
114 complete DDA cycles recorded per chromatographic peak, thus downgrading the quality of peak
115 integration and quantification at the MS level due to insufficient number of data points. This clearly
116 advantages fast MS/MS scanning instruments such as hybrid TOF, with MS acquisition rate up to 100
117 Hz in both the MS and MS/MS modes (at 50K resolution) for the TripleTOF 6600 and timsTOF

118 instruments, compared to the latest generation of quadrupole-Orbitrap instruments (Orbitrap Exploris
119 series) working at 40 Hz at 7.5K resolution at m/z 200 (Perez de Souza et al., 2021).

120 Neumann *et al.* proposed a protocol to realize a better sampling in MS/MS mode by focusing on
121 biologically relevant and meaningful features (Neumann et al., 2013). First, a LC run at the MS level
122 is performed, with the direct analysis of the data by preprocessing software XCMS for automated
123 annotation and statistical analysis to select significant features. Then methods with inclusion lists are
124 created and performed on pooled samples using targeted MS/MS (tMS²) acquisition mode in order to
125 confirm previous annotations while also limiting the number of LC-MS/MS experiments. This method
126 enables a better coverage of the sample by selecting lower abundance relevant features, while losing
127 the unbiased nature of DDA.

128 Koelmel *et al.* developed an Iterative Exclusion IE-Omics approach whose goal is to create several
129 non-redundant inclusion lists (Koelmel et al., 2017). First, a classic DDA acquisition is performed, and
130 all the fragmented ions are added to an exclusion list. The workflow is repeated up to 5 times until the
131 intensity of the precursor ions becomes too low for getting exploitable MS/MS data for automated
132 annotation. When compared to 5 independent DDA scans performed on the same sample, the IE-Omics
133 method shows an increase of 30% in the coverage of the lipidome for a plasma sample.

134 A strategy based on an automated DDA inclusion list workflow, called Data-Set-Dependent
135 Acquisition (DsDA), was developed by Broeckling *et al.* (Broeckling et al., 2018). This method reports
136 the specific combination of MS-data processing and target prioritization to enable a more
137 comprehensive MS/MS coverage of the metabolome. The workflow consists of Top4 DDA scan, then
138 data is automatically treated and a score is attributed to each MS/MS spectra to determine its
139 importance to be fragmented. A module, written in R programming language, forces the workflow to
140 fragment precursor ions not previously fragmented in order to improve the metabolome coverage. This
141 novel method offered 45% more MS/MS features. In the recently introduced BoxCar method, the MS
142 spectrum is segmented in several windows to improve detection sensitivity and the dynamic range of
143 the MS acquisition (Meier et al., 2018). Under these conditions, less abundant ions can be isolated
144 during DDA, thus providing unprecedented structural information.

145 Another recently introduced gas phase fractionation approach is the so-called Parallel Accumulation
146 Serial Fragmentation (PASEF, available on timsTOF Pro instruments) that synchronizes Trapped Ion
147 Mobility Spectrometry (TIMS) with MS/MS precursor selection and fragmentation. This acquisition
148 mode drastically multiplies the fragmentation speed and detection sensitivity thereby approaching
149 almost 100% duty cycle, thus annotating up to 1,100 unique lipids in 1 μ L of human plasma
150 (Vasilopoulou et al., 2020).

151 All the presented methods of DDA are suffering from the same issue. As the sample often needs to be
152 injected several times (up to 5 in both positive and negative ionization modes), its volume needs to be
153 sufficient and the global analysis time is significantly extended making it impossible to apply these
154 strategies for large cohort analysis in the field of medicine or biology.

155 **3 DIA-based acquisition workflows**

156 DIA performance was first described in the proteomic field 18 years ago (Venable et al., 2004) and
157 have been implemented for lipidomics (Schwudke et al., 2006). The application of DIA is essentially
158 motivated by the wider accessible dynamic range (4 orders of magnitude) (Gillet et al., 2012), higher
159 sensitivity and reproducibility and, more importantly, the unbiased characterization of complex
160 biological matrices (Venable et al., 2004).

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161 Analyzing the lipidome by direct injection into the mass spectrometer without prior chromatographic
162 separation steps (shotgun approach) is highly attractive owing to the short acquisition time, the low
163 solvent consumption, and its ease of implementation even if ion suppression effects are usually
164 reported (Schwudke et al., 2011; Yang and Han, 2016). Shotgun DIA mode is mainly based on MS^{ALL}
165 workflow (Simons et al., 2012) and aims at monitoring MS/MS spectral information with 1 Da mass
166 isolation on a large mass range for a run time lasting less than 6 minutes (Simons et al., 2012). MS^{ALL}
167 mode was efficient to identify and quantify lipids belonging to different classes in various biological
168 matrices (Simons et al., 2012; Gao et al., 2016, 2018, 2021).

169 MS^E for Q-TOF or All Ion Fragmentation (AIF) for Orbitrap-based instruments consist in acquiring
170 alternating scans of all ions without mass filtering at two or three collision energies (Plumb et al., 2006)
171 (Figure 1). Precursor information are retrieved at low collision energy whereas specific product ions
172 are given by higher collision energy, facilitating the annotation step by combining all the MS/MS data
173 into consensus MS/MS spectra (Plumb et al., 2006; Diedrich et al., 2013).

174 One of the first approaches of DIA-AIF method was implemented on a LC coupled to a single-stage
175 Orbitrap mass analyzer using polarity switching in a single analytical run and using either in source
176 CID or HCD fragmentation (Gallart-Ayala et al., 2013). Annotation of both phospholipids and
177 triacylglycerols from serum samples for diagnosis of canine mammary cancer thanks to known
178 retention times and typical diagnostic product ions was performed. Similarly, Castro-Perez *et al.*
179 reported the advantages of applying alternated low and high collision energies in positive and negative
180 ionization modes to identify lipids from human plasma (Castro-Perez et al., 2010). More recently, lipids
181 from human plasma and dermal fibroblasts extracts were annotated using DIA-AIF and HCD in both
182 polarities (Ventura et al., 2020a, 2020b).

183 In order to improve the association of precursor ions with their corresponding fragments, SWATH
184 (Sequential Window Acquisition of All THEoretical) has been developed (Gillet et al., 2012). The
185 principle is to define sequential MS/MS experiments in reduced m/z windows, typically 10-20 Da to
186 cover the whole mass range, all ions within these windows are then fragmented simultaneously

187 (Figure 1). It has been initially developed on hybrid TOF instruments since a high-speed acquisition is
188 mandatory, but can be now implemented on Orbitrap-based instruments (Fenaille et al., 2017).
189 SWATH experiments were performed to characterize 214 and 140 lipids from immortalized
190 keratinocytes in both positive and negative ionization modes, respectively. As statistical treatment of
191 the data is mainly based on retention times, authors strongly advise to add internal standards to correct
192 any deviation (Calderón et al., 2020; Cebo et al., 2021). Interestingly, SWATH can be employed for
193 compound annotation using MS/MS library search but also quantification at the MS/MS level because,
194 unlike classic DDA, it offers continuous MS/MS spectra acquisition for particular fragment ions.

195 One of the main drawbacks of DIA workflows is related to the obtained (highly) convoluted tandem
196 mass spectra. Indeed, isobaric or isomeric lipid species from different classes can produce overlapping
197 isotopic clusters that will be co-isolated and fragmented MS/MS thus yielding highly convoluted and
198 thereby difficult-to-match MS/MS spectra. For instance, protonated phosphatidylcholine (PC 33:1) and
199 phosphatidylethanolamine (PE 36:1) share the same elemental composition (Züllig and Köfeler,
200 2021b). The frequent presence of sodium adducts can bring additional spectral complexity with lipid
201 sodiated species isobaric to protonated unsaturated ones differing only by ~2mDa in some extreme
202 cases (Höring et al., 2020; Züllig and Köfeler, 2021b).

203 In DIA workflows, such as SWATH, the direct link between a given precursor ion and its
204 corresponding fragments is often lost, which renders MS/MS data interpretation of unknown lipid
205 species much more difficult than that of MS/MS data resulting from DDA protocols. Data treatment
206 step, i.e. the concatenation and alignment of precursor and fragment ions, is indeed often considered
207 as the critical point. This can be performed according to retention times (Castro-Perez et al., 2010) or
208 by specific data filtering through mass defect calculation (Stagliano et al., 2010). A correlation-based
209 deconvolution (CorrDec) method that uses correlation of ion abundances between precursor and
210 product ions has been developed to improve the metabolite annotation rate (Tada et al., 2020).

211

212 While SWATH techniques provide a better coverage of the lipidome, it comes at the cost of lower
213 quality spectra. Therefore, DIA SWATH method has been implemented together with DDA leading to
214 the hybrid DaDIA mode (Guo et al., 2021). The sequence is composed of blanks and samples analyzed
215 in DIA SWATH mode and quality controls (QCs) in DDA mode. DDA MS/MS data provided high
216 quality spectra for the highest intensity features and are used to improve the deconvolution of SWATH
217 data for better quality. This hybrid method enables a better lipidome coverage while improving the
218 quality of deconvoluted SWATH spectra to a level similar to DDA spectra. Combination of 2D-LC,
219 DIA MS^E and DDA Top3, called HDDIDDA, outperforms DDA with a slightly higher annotation rate
220 and MS^E with a slightly higher coverage at the cost of a complex data treatment (Wang et al., 2022).

221 Even if DIA approach looks appealing in terms of numbers of annotated lipid species, the annotation
222 steps required the most exhaustive and reliable MS/MS databases including normalized retention times
223 if available. Acquiring MS/MS spectra from pure authentic standards is the best way to generate
224 spectral databases, but this is clearly unrealistic due to the cost and viability of pure compounds. The
225 most feasible strategy is to generate *in-silico* low-energy fragmentation spectra thanks to the well-
226 documented mechanisms of lipid fragmentation (Murphy, 2015) as defined in LipidBlast database. As
227 already documented in the literature, MS/MS data are strongly dependent on the type of tandem mass
228 spectrometer and the collision energy used that is challenging to normalize especially in the case of
229 small molecule analysis (Murphy, 2015). Thus, the information related to the relative intensities of the
230 fragments cannot be easily employed to refine structure annotation making the match scoring process
231 less robust.

232 Several software solutions (Kitata et al., 2021) include modules capable of achieving the complete
233 pipeline from the conversion of vendor raw files to statistical analysis and through the identification
234 from integrated and/or implementable lipid libraries as exemplified in Table 1. While attributing ion
235 products to the precursor ion is unproblematic in DDA mode, to reconstruct MS/MS data in DIA mode
236 remains highly challenging. To the best of our knowledge, only two robust software tools, *i.e.* MS-
237 DIAL and DecoMetDIA, offer a deconvolution step for untargeted analysis in the DIA mode (Tsugawa
238 et al., 2019; Yin et al., 2019; Tada et al., 2020). Multiple processing strategies for DIA deconvolution
239 emerge in proteomics (Kitata et al., 2021) and are expected to be applied to small compounds in the
240 near future.

241 **4 Discussion and Conclusions**

242 Considering the rapid development of mass spectrometers in terms of scan speed, resolving power and
243 mass accuracy, the analytical strategies for lipidomics have widely expanded during the last two
244 decades. Beyond the use of high mass accuracy at MS level to suggest molecular formulas, MS/MS
245 experiments are fully integrated in lipidomics workflows to provide successful semi-quantification and

246 structural characterization. Both DDA and DIA methodologies require high acquisition rate tandem
247 mass spectrometers to access deep lipidome. The quality of MS/MS data is needed for facilitating
248 annotation but the total number of MS/MS scans on a unique feature is limited. Iterative DDA allows
249 to exceed this limitation but significantly increases the sample consumption and the overall
250 experimental time making this approach not compatible with the analysis of large cohorts. On the
251 contrary, DIA significantly increases the number of annotated lipid species but is still far limited by
252 the available software solutions (notably regarding spectra deconvolution).

253 Free software tools are reviewed in the Table 1 and three of them, namely MS-DIAL (Tsugawa et al.,
254 2015), DecoMetDIA (Yin et al., 2019) and DecoID (Stancliffe et al., 2021) propose efficient
255 bioinformatic tools for the deconvolution of mixed tandem mass spectra obtained from SWATH
256 workflows.

257 Also, as exposed by Barbier Saint Hilaire *et al.*, well-designing SWATH isolation windows can impact
258 significantly the detection rate of targeted compounds (Barbier Saint Hilaire et al., 2020). The free
259 software tool SwathTUNER (Table 1) permits the automatized design of the variable isolations
260 windows according either the Total Ion Current (TIC) or precursors density (Zhang et al., 2015) to
261 significantly lower the complexity of tandem mass spectra obtained in the DIA-SWATH mode. Also,
262 the commercial vendor Sciex proposes the SWATH® Acquisition module available on their
263 TripleTOF® systems for the automatically designing of variable isolation windows, permitting the all-
264 in-one acquisition workflow (<https://sciex.com/technology/swath-acquisition>).

265 Since a decade, ion mobility (IM) offers the unique possibility to separate ions not only on their m/z
266 values but also on their shape in the gas phase, thus enabling the measure of collision cross-sections
267 (CCSs). The IM time is fully compatible with the chromatographic time and the fast acquisition time
268 of modern mass spectrometers. Several groups demonstrated that lipid isomers bearing different fatty
269 chains but the same total number of carbons, including Z/E isomers, can be efficiently separated by
270 IM-MS (Paglia et al., 2015). MS^E experiments by coupling LC to IM-QTOF were performed for the
271 characterization of non-purified glycolipids extracts from *Caenorhabditis elegans* (Witting et al.,
272 2021). The multidimensional information collected from chromatographic separation (Retention
273 Time), ion mobility (CCS) and MS^E acquisition permits extended lipid annotation. Ion mobility
274 coupled to DIA or DDA approaches paves the way for a rather complete structural annotation and
275 (semi-)quantification of deep lipidome including all minor species, while also significantly improving
276 the quality of both DDA and DIA tandem mass spectra by separating isobaric/isomeric lipid ion species
277 prior to their isolation and fragmentation. The file sizes, from several hundreds of Mo to several Go
278 per injection, the limited software availability for efficiently handling resulting datasets and the
279 difficulty to generate absolute collision cross-sections for highly flexible molecules, such as lipids in
280 the gas phase, strongly restrict the full exploitation of the data acquired by DIA- or DDA- ion mobility.

281 A last issue in MS/MS is the efficient location of double bonds on lipid structures as dissociations at
282 low-energy are not efficient (Ma et al., 2016). Prior chemical derivatization of carbon-carbon double
283 bonds either directly by a chemical agent (e.g. ozonolysis, epoxidation, hydroxylation) or facilitated
284 by UV irradiation (e.g. Paternò – Büchi reaction) can lead to reaction products that can be further
285 fragmented under low-energy dissociation conditions to pinpoint the location of double bonds (Cao et
286 al., 2018; Harris et al., 2018). Nonetheless, the routine and broad application of these methods remain
287 limited due to the required long reaction time and the inadequate utilization with online LC-MS/MS
288 workflows (Poad et al., 2017). Other fragmentation techniques can be also implemented. Electron-
289 capture Dissociation (ECD) on a divalent metal complex of glycerophospholipids was proposed for
290 double bond location (James et al., 2011). Other electron-activated dissociation (EAD) types have been

291 devised such as Electron-induced dissociation (EID) and Electron-Impact Excitation of Ions from
292 Organics (EIEIO) compatible with singly-charged precursor ions (Baba et al., 2021). Ultraviolet
293 photodissociation (UVPD) activation mode has recently been used to distinguish sn-positional isomers
294 and to determine carbon-carbon double bonds location (Williams et al., 2017). Only few commercial
295 instruments are now equipped with EAD, EID, EIEO or UVPD while relatively low yields of
296 fragmentation are usually reported for those methods restricting their applications to abundant species
297 even if the fragmentation process is fully compatible with LC peak width.

298 Even if major progresses in lipidomics have been reported in the last years, improvements of each step
299 of the workflow, from the sample preparation, the chromatography, the MS and MS/MS acquisition to
300 the automated data treatment, are needed to expand lipidome coverage.

301 **5 Author Contributions**

302 VM and MV equally contributed to the work. All authors completed and approved the manuscript.

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307 **7 References**

308 Alcoriza-Balaguer, M. I., Garcia-Cañaveras, J. C., López, A., Conde, I., Juan, O., Carretero, J., et al.
309 (2019). LipidMS: An R Package for Lipid Annotation in Untargeted Liquid Chromatography-
310 Data Independent Acquisition-Mass Spectrometry Lipidomics. *Anal. Chem.* 91(1), 836–845.
311 doi: 10.1021/acs.analchem.8b03409.

312 Baba, T., Ryumin, P., Duchoslav, E., Chen, K., Chelur, A., Loyd, B., et al. (2021). Dissociation of
313 Biomolecules by an Intense Low-Energy Electron Beam in a High Sensitivity Time-of-Flight
314 Mass Spectrometer. *J. Am. Soc. Mass Spectrom.* 32(8), 1964–1975. doi:
315 10.1021/jasms.0c00425.

316 Barbier Saint Hilaire, P., Rousseau, K., Seyer, A., Dechaumet, S., Damont, A., Junot, C., et al. (2020).
317 Comparative Evaluation of Data Dependent and Data Independent Acquisition Workflows
318 Implemented on an Orbitrap Fusion for Untargeted Metabolomics. *Metabolites* 10, 158. doi:
319 10.3390/metabo10040158.

320 Broeckling, C. D., Hoyes, E., Richardson, K., Brown, J. M., and Prenni, J. E. (2018). Comprehensive
321 Tandem-Mass-Spectrometry Coverage of Complex Samples Enabled by Data-Set-Dependent
322 Acquisition. *Anal. Chem.* 90, 8020–8027. doi: 10.1021/acs.analchem.8b00929.

323 Byrdwell, W. C. (2001). Atmospheric pressure chemical ionization mass spectrometry for analysis of
324 lipids. *Lipids* 36(4), 327–346. doi: 10.1007/s11745-001-0725-5.

325 Calderón, C., Rubarth, L., Cebo, M., Merfort, I., and Lämmerhofer, M. (2020). Lipid Atlas of
326 Keratinocytes and Betulin Effects on its Lipidome Profiled by Comprehensive UHPLC–

- 327 MS/MS with Data Independent Acquisition Using Targeted Data Processing. *PROTEOMICS*
328 20(11):e1900113. doi: 10.1002/pmic.201900113.
- 329 Cao, W., Ma, X., Li, Z., Zhou, X., and Ouyang, Z. (2018). Locating Carbon–Carbon Double Bonds in
330 Unsaturated Phospholipids by Epoxidation Reaction and Tandem Mass Spectrometry. *Anal.*
331 *Chem.* 90(17), 10286–10292. doi: 10.1021/acs.analchem.8b02021.
- 332 Castro-Perez, J. M., Kamphorst, J., DeGroot, J., Lafeber, F., Goshawk, J., Yu, K., et al. (2010).
333 Comprehensive LC–MSE Lipidomic Analysis using a Shotgun Approach and Its Application
334 to Biomarker Detection and Identification in Osteoarthritis Patients. *J. Proteome Res.* 9(5),
335 2377–2389. doi: 10.1021/pr901094j.
- 336 Cebo, M., Calderón Castro, C., Schlotterbeck, J., Gawaz, M., Chatterjee, M., and Lämmerhofer, M.
337 (2021). Untargeted UHPLC-ESI-QTOF-MS/MS analysis with targeted feature extraction at
338 precursor and fragment level for profiling of the platelet lipidome with ex vivo thrombin-
339 activation. *J. Pharm. Biomed. Anal.* 205:114301. doi: 10.1016/j.jpba.2021.114301.
- 340 Chiu, H.-H., and Kuo, C.-H. (2020). Gas chromatography-mass spectrometry-based analytical
341 strategies for fatty acid analysis in biological samples. *Journal of Food and Drug Analysis* 28,
342 60–73. doi: 10.1016/j.jfda.2019.10.003.
- 343 Diedrich, J. K., Pinto, A. F. M., and Yates, J. R. (2013). Energy dependence of HCD on peptide
344 fragmentation: Stepped collisional energy finds the sweet spot. *J. Am. Soc. Mass Spectrom.*
345 24(11), 1690–1699. doi: 10.1007/s13361-013-0709-7.
- 346 Eliuk, S., and Makarov, A. (2015). Evolution of Orbitrap Mass Spectrometry Instrumentation. *Annu.*
347 *Rev. Anal. Chem.* 8(1), 61–80. doi: 10.1146/annurev-anchem-071114-040325.
- 348 Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Murphy, R. C., et al. (2005). A
349 comprehensive classification system for lipids. *J Lipid Res* 46, 839–861. doi:
350 10.1194/jlr.E400004-JLR200.
- 351 Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R. H., Shimizu, T., et al. (2009).
352 Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* 50, S9–
353 S14. doi: 10.1194/jlr.R800095-JLR200.
- 354 Fenaille, F., Barbier Saint-Hilaire, P., Rousseau, K., and Junot, C. (2017). Data acquisition workflows
355 in liquid chromatography coupled to high resolution mass spectrometry-based metabolomics:
356 Where do we stand? *J. Chromatogr. A* 1526, 1–12. doi: 10.1016/j.chroma.2017.10.043.
- 357 Gallart-Ayala, H., Courant, F., Severe, S., Antignac, J.-P., Morio, F., Abadie, J., et al. (2013). Versatile
358 lipid profiling by liquid chromatography–high resolution mass spectrometry using all ion
359 fragmentation and polarity switching. Preliminary application for serum samples phenotyping
360 related to canine mammary cancer. *Anal. Chim. Acta* 796, 75–83. doi:
361 10.1016/j.aca.2013.08.006.
- 362 Gao, F., McDaniel, J., Chen, E. Y., Rockwell, H. E., Nguyen, C., Lynes, M. D., et al. (2018). Adapted
363 MS/MSALL Shotgun Lipidomics Approach for Analysis of Cardiolipin Molecular Species.
364 *Lipids* 53(1), 133–142. doi: 10.1002/lipd.12004.

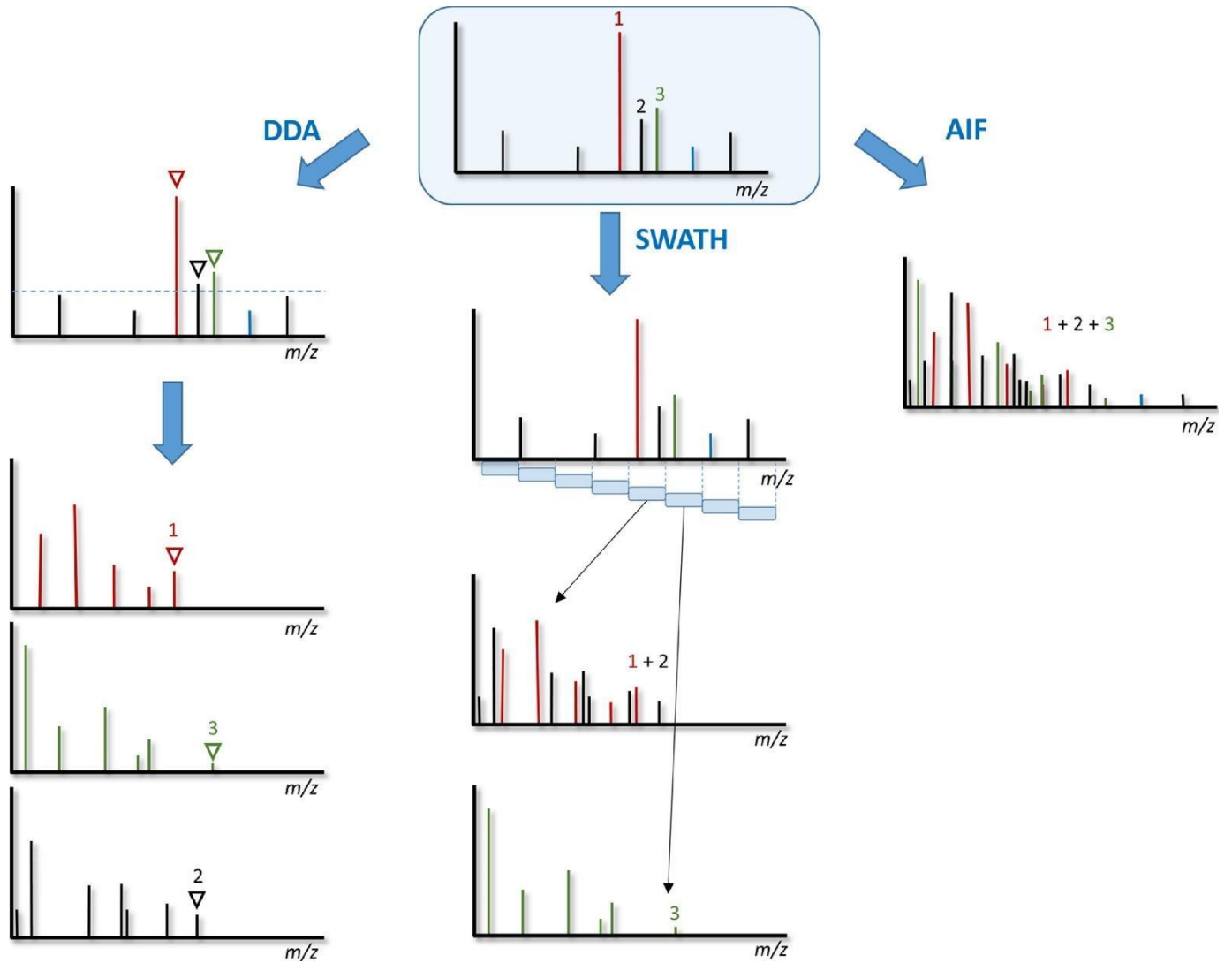
- 365 Gao, F., McDaniel, J., Chen, E. Y., Rockwell, H., Lynes, M. D., Tseng, Y.-H., et al. (2016).
366 Monoacylglycerol Analysis Using MS/MSALL Quadruple Time of Flight Mass Spectrometry.
367 *Metabolites* 6(3):25. doi: 10.3390/metabo6030025.
- 368 Gao, Y., Qi, Y., Huang, Y., Li, X., Zhou, L., and Zhao, S. (2021). Lipidomics Analysis of the Tears in
369 the Patients Receiving LASIK, FS-LASIK, or SBK Surgery. *Front. Med.* 8:731462. doi:
370 10.3389/fmed.2021.731462.
- 371 Gillet, L. C., Navarro, P., Tate, S., Röst, H., Selevsek, N., Reiter, L., et al. (2012). Targeted Data
372 Extraction of the MS/MS Spectra Generated by Data-independent Acquisition: A New Concept
373 for Consistent and Accurate Proteome Analysis*. *Mol. Cell. Proteomics* 11(6), O111.016717.
374 doi: 10.1074/mcp.O111.016717.
- 375 Goracci, L., Tortorella, S., Tiberi, P., Pellegrino, R. M., Di Veroli, A., Valeri, A., et al. (2017). Lipostar,
376 a Comprehensive Platform-Neutral Cheminformatics Tool for Lipidomics. *Anal. Chem.* 89(11),
377 6257–6264. doi: 10.1021/acs.analchem.7b01259.
- 378 Guo, J., Shen, S., Xing, S., and Huan, T. (2021). DaDIA: Hybridizing Data-Dependent and Data-
379 Independent Acquisition Modes for Generating High-Quality Metabolomic Data. *Anal. Chem.*
380 93, 2669–2677. doi: 10.1021/acs.analchem.0c05022.
- 381 Harayama, T., and Riezman, H. (2018). Understanding the diversity of membrane lipid composition.
382 *Nat Rev Mol Cell Biol* 19, 281–296. doi: 10.1038/nrm.2017.138.
- 383 Harris, R. A., May, J. C., Stinson, C. A., Xia, Y., and McLean, J. A. (2018). Determining Double Bond
384 Position in Lipids Using Online Ozonolysis Coupled to Liquid Chromatography and Ion
385 Mobility-Mass Spectrometry. *Anal. Chem.* 90(3), 1915–1924. doi:
386 10.1021/acs.analchem.7b04007.
- 387 Hartler, J., Triebel, A., Ziegl, A., Trötz Müller, M., Rechberger, G. N., Zeleznik, O. A., et al. (2017).
388 Deciphering lipid structures based on platform-independent decision rules. *Nat Methods*
389 14(12), 1171–1174. doi: 10.1038/nmeth.4470.
- 390 Hartler, J., Trötz Müller, M., Chittraju, C., Spener, F., Köfeler, H. C., and Thallinger, G. G. (2011). Lipid
391 Data Analyzer: unattended identification and quantitation of lipids in LC-MS data.
392 *Bioinformatics* 27(4), 572–577. doi: 10.1093/bioinformatics/btq699.
- 393 Höring, M., Ekroos, K., Baker, P. R. S., Connell, L., Stadler, S. C., Burkhardt, R., et al. (2020).
394 Correction of Isobaric Overlap Resulting from Sodiated Ions in Lipidomics. *Anal. Chem.* 92,
395 10966–10970. doi: 10.1021/acs.analchem.0c02408.
- 396 Hutchins, P. D., Russell, J. D., and Coon, J. J. (2018). LipiDex: An Integrated Software Package for
397 High-Confidence Lipid Identification. *cels* 6(5), 621-625.e5. doi: 10.1016/j.cels.2018.03.011.
- 398 Imbert, L., Gaudin, M., Libong, D., Touboul, D., Abreu, S., Loiseau, P. M., et al. (2012). Comparison
399 of electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure
400 photoionization for a lipidomic analysis of *Leishmania donovani*. *J. Chromatogr. A* 1242, 75–
401 83. doi: 10.1016/j.chroma.2012.04.035.

- 402 James, P. F., Perugini, M. A., and O’Hair, R. A. J. (2011). Electron capture dissociation of complexes
403 of diacylglycerophosphocholine and divalent metal ions: Competition between charge
404 reduction and radical induced phospholipid fragmentation. *J. Am. Soc. Mass Spectrom.* 19,
405 978–986. doi: 10.1016/j.jasms.2008.03.006.
- 406 Kirkwood, K. I., Pratt, B. S., Shulman, N., Tamura, K., MacCoss, M. J., MacLean, B. X., et al. (2022).
407 Utilizing Skyline to analyze lipidomics data containing liquid chromatography, ion mobility
408 spectrometry and mass spectrometry dimensions. *Nat Protoc*, 1–16. doi: 10.1038/s41596-022-
409 00714-6.
- 410 Kitata, R. B., Yang, J.-C., and Chen, Y.-J. (2021). Advances in data-independent acquisition mass
411 spectrometry towards comprehensive digital proteome landscape. *Mass Spectrom. Rev.*,
412 e21781. doi: 10.1002/mas.21781.
- 413 Kochen, M. A., Chambers, M. C., Holman, J. D., Nesvizhskii, A. I., Weintraub, S. T., Belisle, J. T., et
414 al. (2016). Greazy: Open-Source Software for Automated Phospholipid Tandem Mass
415 Spectrometry Identification. *Anal. Chem.* 88, 5733–5741. doi: 10.1021/acs.analchem.6b00021.
- 416 Koelmel, J. P., Kroeger, N. M., Gill, E. L., Ulmer, C. Z., Bowden, J. A., Patterson, R. E., et al. (2017).
417 Expanding lipidome coverage using LC-MS/MS data-dependent acquisition with automated
418 exclusion list generation. *J Am Soc Mass Spectrom* 28, 908–917. doi: 10.1007/s13361-017-
419 1608-0.
- 420 Li, H., Cai, Y., Guo, Y., Chen, F., and Zhu, Z.-J. (2016). MetDIA: Targeted Metabolite Extraction of
421 Multiplexed MS/MS Spectra Generated by Data-Independent Acquisition. *Anal. Chem.* 88,
422 8757–8764. doi: 10.1021/acs.analchem.6b02122.
- 423 Liebisch, G., Fahy, E., Aoki, J., Dennis, E. A., Durand, T., Ejsing, C. S., et al. (2020). Update on LIPID
424 MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. *J.*
425 *Lipid Res.* 61(12), 1539–1555. doi: 10.1194/jlr.S120001025.
- 426 Ma, X., Chong, L., Tian, R., Shi, R., Hu, T. Y., Ouyang, Z., et al. (2016). Identification and quantitation
427 of lipid C=C location isomers: A shotgun lipidomics approach enabled by photochemical
428 reaction. *Proc. Natl. Acad. Sci.* 113(10), 2573–2578. doi: 10.1073/pnas.1523356113.
- 429 Meier, F., Geyer, P. E., Virreira Winter, S., Cox, J., and Mann, M. (2018). BoxCar acquisition method
430 enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes. *Nat Methods* 15,
431 440–448. doi: 10.1038/s41592-018-0003-5.
- 432 Murphy, R. C. (2015). *Tandem mass spectrometry of lipids: molecular analysis of complex lipids.*
433 Cambridge: Royal Society of Chemistry.
- 434 Neumann, S., Thum, A., and Böttcher, C. (2013). Nearline acquisition and processing of liquid
435 chromatography-tandem mass spectrometry data. *Metabolomics* 9, 84–91. doi:
436 10.1007/s11306-012-0401-0.
- 437 Paglia, G., Angel, P., Williams, J. P., Richardson, K., Olivos, H. J., Thompson, J. W., et al. (2015). Ion
438 Mobility-Derived Collision Cross Section As an Additional Measure for Lipid Fingerprinting
439 and Identification. *Anal Chem* 87, 1137–1144. doi: 10.1021/ac503715v.

- 440 Perez de Souza, L., Alseekh, S., Scossa, F., and Fernie, A. R. (2021). Ultra-high-performance liquid
441 chromatography high-resolution mass spectrometry variants for metabolomics research. *Nat*
442 *Methods* 18(7), 733–746. doi: 10.1038/s41592-021-01116-4.
- 443 Plumb, R. S., Johnson, K. A., Rainville, P., Smith, B. W., Wilson, I. D., Castro-Perez, J. M., et al.
444 (2006). UPLC/MSE; a new approach for generating molecular fragment information for
445 biomarker structure elucidation. *Rapid Communications in Mass Spectrometry* 20(13), 1989–
446 1994. doi: 10.1002/rcm.2550.
- 447 Poad, B. L. J., Green, M. R., Kirk, J. M., Tomczyk, N., Mitchell, T. W., and Blanksby, S. J. (2017).
448 High-Pressure Ozone-Induced Dissociation for Lipid Structure Elucidation on Fast
449 Chromatographic Timescales. *Anal. Chem.* 89(7), 4223–4229. doi:
450 10.1021/acs.analchem.7b00268.
- 451 Quehenberger, O., Armando, A. M., Brown, A. H., Milne, S. B., Myers, D. S., Merrill, A. H., et al.
452 (2010). Lipidomics reveals a remarkable diversity of lipids in human plasma. *Journal of Lipid*
453 *Research* 51, 3299–3305. doi: 10.1194/jlr.M009449.
- 454 Rustam, Y. H., and Reid, G. E. (2018). Analytical Challenges and Recent Advances in Mass
455 Spectrometry Based Lipidomics. *Anal. Chem.* 90(1), 374–397. doi:
456 10.1021/acs.analchem.7b04836.
- 457 Schwudke, D., Oegema, J., Burton, L., Entchev, E., Hannich, J. T., Ejsing, C. S., et al. (2006). Lipid
458 Profiling by Multiple Precursor and Neutral Loss Scanning Driven by the Data-Dependent
459 Acquisition. *Anal. Chem.* 78(2), 585–595. doi: 10.1021/ac051605m.
- 460 Schwudke, D., Schuhmann, K., Herzog, R., Bornstein, S. R., and Shevchenko, A. (2011). Shotgun
461 lipidomics on high resolution mass spectrometers. *Cold Spring Harb Perspect Biol* 3, a004614.
462 doi: 10.1101/cshperspect.a004614.
- 463 Simons, B., Kauhanen, D., Sylvänne, T., Tarasov, K., Duchoslav, E., and Ekroos, K. (2012). Shotgun
464 Lipidomics by Sequential Precursor Ion Fragmentation on a Hybrid Quadrupole Time-of-Flight
465 Mass Spectrometer. *Metabolites* 2(1), 195–213. doi: 10.3390/metabo2010195.
- 466 Stagliano, M. C., DeKeyser, J. G., Omiecinski, C. J., and Jones, A. D. (2010). Bioassay-directed
467 fractionation for discovery of bioactive neutral lipids guided by relative mass defect filtering
468 and multiplexed collision-induced dissociation. *Rapid Commun. Mass Spectrom.* 24(24), 3578–
469 3584. doi: 10.1002/rcm.4796.
- 470 Stancliffe, E., Schwaiger-Haber, M., Sindelar, M., and Patti, G. J. (2021). DecoID Improves
471 Identification Rates in Metabolomics through Database-Assisted MS/MS Deconvolution. *Nat*
472 *Methods* 18, 779–787. doi: 10.1038/s41592-021-01195-3.
- 473 Tada, I., Chaleckis, R., Tsugawa, H., Meister, I., Zhang, P., Lazarinis, N., et al. (2020). Correlation-
474 Based Deconvolution (CorrDec) To Generate High-Quality MS2 Spectra from Data-
475 Independent Acquisition in Multisample Studies. *Anal. Chem.* 92(16), 11310–11317. doi:
476 10.1021/acs.analchem.0c01980.

- 477 Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., et al. (2015). MS-DIAL: data-
478 independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat. Methods*
479 12(6), 523–526. doi: 10.1038/nmeth.3393.
- 480 Tsugawa, H., Satoh, A., Uchino, H., Cajka, T., Arita, M., and Arita, M. (2019). Mass Spectrometry
481 Data Repository Enhances Novel Metabolite Discoveries with Advances in Computational
482 Metabolomics. *Metabolites* 9, 119. doi: 10.3390/metabo9060119.
- 483 Vasilopoulou, C. G., Sulek, K., Brunner, A.-D., Meitei, N. S., Schweiger-Hufnagel, U., Meyer, S. W.,
484 et al. (2020). Trapped ion mobility spectrometry and PASEF enable in-depth lipidomics from
485 minimal sample amounts. *Nat Commun* 11(1), 331. doi: 10.1038/s41467-019-14044-x.
- 486 Venable, J. D., Dong, M.-Q., Wohlschlegel, J., Dillin, A., and Yates, J. R. (2004). Automated approach
487 for quantitative analysis of complex peptide mixtures from tandem mass spectra. *Nat. Methods*
488 1(1), 39–45. doi: 10.1038/nmeth705.
- 489 Ventura, G., Bianco, M., Calvano, C. D., Losito, I., and Cataldi, T. R. I. (2020a). HILIC-ESI-FTMS
490 with All Ion Fragmentation (AIF) Scans as a Tool for Fast Lipidome Investigations. *Molecules*
491 25(10):2310. doi: 10.3390/molecules25102310.
- 492 Ventura, G., Calvano, C. D., Porcelli, V., Palmieri, L., De Giacomo, A., Xu, Y., et al. (2020b).
493 Phospholipidomics of peripheral blood mononuclear cells (PBMCs): the tricky case of children
494 with autism spectrum disorder (ASD) and their healthy siblings. *Anal. Bioanal. Chem.* 412(25),
495 6859–6874. doi: 10.1007/s00216-020-02817-z.
- 496 Wang, H., Wang, H., Wang, X., Xu, X., Hu, Y., Li, X., et al. (2022). A novel hybrid scan approach
497 enabling the ion-mobility separation and the alternate data-dependent and data-independent
498 acquisitions (HDDIDDA): Its combination with off-line two-dimensional liquid
499 chromatography for comprehensively characterizing the multicomponents from Compound
500 Danshen Dripping Pill. *Analytica Chimica Acta* 1193, 339320. doi: 10.1016/j.aca.2021.339320.
- 501 Wenk, M. R. (2005). The emerging field of lipidomics. *Nat Rev Drug Discov* 4(7), 594–610. doi:
502 10.1038/nrd1776.
- 503 Wenk, M. R. (2010). Lipidomics: New Tools and Applications. *Cell* 143(6), 888–895. doi:
504 10.1016/j.cell.2010.11.033.
- 505 Williams, P. E., Klein, D. R., Greer, S. M., and Brodbelt, J. S. (2017). Pinpointing Double Bond and
506 sn-Positions in Glycerophospholipids via Hybrid 193 nm Ultraviolet Photodissociation
507 (UVPD) Mass Spectrometry. *J. Am. Chem. Soc.* 139(44), 15681–15690. doi:
508 10.1021/jacs.7b06416.
- 509 Witting, M., Schmidt, U., and Knoelker, H.-J. (2021). UHPLC-IM-Q-ToFMS analysis of maradolipids,
510 found exclusively in *Caenorhabditis elegans* dauer larvae. *Anal. Bioanal. Chem.* 413(8), 2091–
511 2102. doi: 10.1007/s00216-021-03172-3.
- 512 Yang, K., and Han, X. (2016). Lipidomics: Techniques, applications, and outcomes related to
513 biomedical sciences. *Trends Biochem Sci* 41, 954–969. doi: 10.1016/j.tibs.2016.08.010.

- 514 Yin, Y., Wang, R., Cai, Y., Wang, Z., and Zhu, Z.-J. (2019). DecoMetDIA: Deconvolution of
515 Multiplexed MS/MS Spectra for Metabolite Identification in SWATH-MS-Based Untargeted
516 Metabolomics. *Anal. Chem.* 91(18), 11897–11904. doi: 10.1021/acs.analchem.9b02655.
- 517 Zha, H., Cai, Y., Yin, Y., Wang, Z., Li, K., and Zhu, Z.-J. (2018). SWATHtoMRM: Development of
518 High-Coverage Targeted Metabolomics Method Using SWATH Technology for Biomarker
519 Discovery. *Anal. Chem.* 90, 4062–4070. doi: 10.1021/acs.analchem.7b05318.
- 520 Zhang, Y., Bilbao, A., Bruderer, T., Luban, J., Strambio-De-Castillia, C., Lisacek, F., et al. (2015). The
521 Use of Variable Q1 Isolation Windows Improves Selectivity in LC–SWATH–MS Acquisition.
522 *J. Proteome Res.* 14(10), 4359–4371. doi: 10.1021/acs.jproteome.5b00543.
- 523 Zhou, Z., Shen, X., Chen, X., Tu, J., Xiong, X., and Zhu, Z.-J. (2019). LipidIMMS Analyzer:
524 integrating multi-dimensional information to support lipid identification in ion mobility—mass
525 spectrometry based lipidomics. *Bioinformatics* 35(4), 698–700. doi:
526 10.1093/bioinformatics/bty661.
- 527 Züllig, T., and Köfeler, H. C. (2021a). High Resolution Mass Spectrometry in Lipidomics. *Mass*
528 *Spectrom. Rev.* 40(3), 162–176. doi: 10.1002/mas.21627.
- 529 Züllig, T., and Köfeler, H. C. (2021b). HIGH RESOLUTION MASS SPECTROMETRY IN
530 LIPIDOMICS. *Mass Spectrom Rev* 40, 162–176. doi: 10.1002/mas.21627.
- 531 Züllig, T., Trötz Müller, M., and Köfeler, H. C. (2020). Lipidomics from sample preparation to data
532 analysis: a primer. *Anal. Bioanal. Chem.* 412(10), 2191–2209. doi: 10.1007/s00216-019-
533 02241-y.
- 534



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537 Figure 1: Scheme illustrating the DDA, SWATH and AIF acquisition workflows reproduced with the
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Table 1 - Overview of software and tools for the processing of lipid identification step from spectral data acquired by tandem mass spectrometry in DDA and DIA. Remark: Software specified by * are commercial.

Software Tools	Data processing			Lipid annotation		Statistical tools	Application and descriptions
	Peak detection	Peak alignment	Peak grouping	Precursor match	MS/MS match		
Lipid Search*	X	X	X	X	X	X	DDA: ESI-(LC)-MS/MS, identification from integrated lipid library, univariate statistics and MS/MS scoring. By Thermo Scientific™.
LipidView*, Lipidizer*	X	X	X	X	X	X	DDA: ESI-(LC)-MS/MS, identification from integrated lipid library, univariate statistics and MS/MS scoring. By SCIEX.
SimLipid*	X	X	X	X	X	X	DDA, DIA: ESI-(LC)-MS/MS identification from integrated lipid library, univariate statistical analysis and MS/MS scoring (MS ^E for DIA mode acquisition). By PREMIER Biosoft.
Lipostar*	X	X	X	X	X	X	DDA, DIA: ESI-LC-MS/MS identification from implementable lipid library, multivariate unsupervised and supervised statistical analyses (e.g. Principal Component Analysis (PCA), Partial Least-Squares (PLS)) (Goracci et al., 2017). By Molecular Discovery.
SwathTuner							DIA: Tool to tune method and optimize width windows for SWATH acquisitions (Zhang et al., 2015).
MS-DIAL	X	X	X	X	X	X	DDA, DIA: Deconvolution of MS/MS spectra of SWATH acquisition (two available algorithms) and identification from implementable lipid libraries with MS/MS scoring. Univariate and non-supervised statistics (e.g. T-test, PCA) (Tsubawa et al., 2015; Tada et al., 2020).
MetDIA	X	X	X	X	X	X	DIA: Targeted peak detection from SWATH acquisition according to implementable lipid libraries with MS/MS scoring, univariate statistics on precursors ion area (Li et al., 2016).
Lipidex	X	X	X	X	X		DDA: Peak detection and identification from implementable lipid libraries (Hutchins et al., 2018).
LipidMS 3.0	X	X	X	X	X		DDA, DIA: Targeted peak detection from the integrated lipid libraries (Alcoriza-Balaguer et al., 2019).
Skyline	X	X	X	X	X		DDA, DIA: Targeted approach for identification according indexed Retention Time and Collision-Cross Section, MS and MS/MS spectra from implementable lipid libraries (Kirkwood et al., 2022).
DecoMetDIA	X	X	X				DIA: Deconvolution of MS/MS spectra from SWATH acquisitions and identification according to implementable lipid libraries (Yin et al., 2019).
Greazy/LipidLama	X		X	X	X		DDA: Peak detection and identification from set of theoretical phospholipids and fragments (Kochen et al., 2016).
DecoID				X	X		DDA, DIA: Peak detection, deconvolution of MS/MS spectra (LASSO regression algorithm) and identification according implementable lipid libraries (Stancliffe et al., 2021).
SWATHtoMRM				X	X		DIA: Targeted peak detection from SWATH acquisitions from implementable lipid libraries (Zha et al., 2018).
Lipid Data Analyzer2 (LDA2)				X	X		DDA, DIA: Semi-targeted approach for identification according to decision rules from various mass spectrometers (Hartler et al., 2011, 2017).
LipidMatch				X	X		DDA, DIA: Untargeted approach for identification according <i>in-silico</i> lipid libraries. For DIA, AIF uniquely (Koelmel et al., 2017).
LipidIMMS				X	X		DDA, DIA: Targeted approach for identification according Retention Time and Collision-Cross Section predictions, MS and MS/MS spectra from integrated lipid libraries (Zhou et al., 2019).

