Applications of Fourier Transform Ion Cyclotron Resonance (FT-ICR) and Orbitrap Based High Resolution Mass Spectrometry in Metabolomics and Lipidomics

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Abstract: Metabolomics, along with other “omics” approaches, is rapidly becoming one of the major approaches aimed at understanding the organization and dynamics of metabolic networks. Mass spectrometry is often a technique of choice for metabolomics studies due to its high sensitivity, reproducibility and wide dynamic range. High resolution mass spectrometry (HRMS) is a widely practiced technique in analytical and bioanalytical sciences. It offers exceptionally high resolution and the highest degree of structural confirmation. Many metabolomics studies have been conducted using HRMS over the past decade. In this review, we will explore the latest developments in Fourier transform mass spectrometry (FTMS) and Orbitrap based metabolomics technology, its advantages and drawbacks for using in metabolomics and lipidomics studies, and development of novel approaches for processing HRMS data.

Keywords: high resolution mass spectrometry; metabolomics; lipidomics; FTMS; FT-ICR-MS; Orbitrap-MS; metabolomics data analysis

1. Introduction

Metabolomics is a global approach aimed at measuring cell metabolomes, which are “context dependent, varying according to the physiology, developmental or pathological state of the cell, tissue, organ or organism” [1]. Metabolomics experiments generally target a large number of chemically diverse small molecular weight compounds including primary metabolites, such as organic acids, amino acids, sugars, sugar alcohols, sugar phosphates, amines, fatty acids, polar lipids, hormones and vitamins, as well as specialized metabolites, like phenolics, flavonoids, monoterpenes, sesquiterpenes, polyketides, alkaloids, and others. Lipidomics aims at measuring a full complement of lipid molecular species in cells, tissues, or organisms [2]. There is an overlap in metabolites usually covered by metabolomics and lipidomics (i.e., many polar lipids, fatty acids, eicosanoids and fat soluble vitamins), and some scientists consider lipids as a subset of metabolome and lipidomics as part of metabolomics. In recent years, metabolomics and lipidomics have become the major analytical approaches in many areas of biology ranging from studying gene functions to systems biology research complementing genomics, transcriptomics and proteomics approaches aimed at understanding global state of the cell.

Mass spectrometry (MS) is often the technique of choice to generate high-throughput metabolomics and lipidomics data due to high sensitivity, relatively short analysis time, wide dynamic range, high reproducibility and, most importantly, its ability to analyze samples with extreme molecular complexity [3–6]. Over the years, MS has proven itself as powerful technology for the detection
and annotation of diverse metabolite classes and has become an important tool for metabolomics analysis in numerous organisms [7]. Therefore, various conventional MS based multiclass analyses are now being replaced by metabolomics approaches that offer excellent combinations of analytical and bioinformatics tools and can provide comprehensive information on a large number of metabolites in any particular system.

Fourier transform mass spectrometers (FTMS) or Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR-MS) are the most advanced mass analyzers in terms of high accuracy and resolving power with sub-parts-per-million mass accuracy [8]. The FT based mass analyzers principally use cyclotron frequency in the fixed magnetic field for the determination of the ions mass to charge ratio (m/z) [9,10] and provide the resolution and mass accuracy that are required to reliably assign molecular formulas to detected ions [11]. The accurate mass measurement by FTMS has been widely demonstrated for the characterization of unknown metabolites by the unambiguous assignment of elemental formulas [3,10,12–15]. These characteristics of FTMS are ideal for the types of complex mixtures encountered in high throughput metabolomics applications [13].

The disadvantage of FT-ICR-MS instruments is their relatively slow acquisition rates. At a scan rate of 1 Hz with mass resolution of 100,000 at m/z 4000, the number of points over the chromatographic peak, especially if additional MS/MS scans are required, is low when FTMS is combined with modern fast chromatography systems. This limits the application of FT-ICR-MS in liquid chromatography mass spectrometry (LC-MS) and capillary electrophoresis mass spectrometry (CE-MS) based metabolomics. Introduction of the Orbitrap mass analyzer [16] and coupling Orbitrap with liquid [17] and later gas [18,19] chromatography resulted in a growing number of studies employing high resolution mass spectrometry (HRMS) in metabolomics and lipidomics.

Metabolomics [13,20,21] and lipidomics [22] applications of FT-ICR-MS and Orbitrap-MS have been previously reviewed. In this short review, we provide a critical overview of the latest developments in the HRMS based metabolomics approach and its potential for metabolomics, lipidomics and high-density pharmaceutical and environmental analysis. We will mostly focus on applications of FT-ICR-MS and Orbitrap instruments.

2. Advantages of Fourier Transform Ion Cyclotron Resonance (FT-ICR) and Orbitrap Based High Resolution Mass Spectrometry (HRMS) for Metabolomics and Lipidomics

High Resolution Mass Spectrometers can routinely achieve mass accuracy below 5 ppm and a mass resolution above 10,000 (which is ratio of measured mass (m) to theoretical mass (Δm) m/Δm, at the full width of the peak at half of its maximum height (FWHM)) (reviewed by [20]). Mass analyzers that can perform HRMS are FT-ICR, Orbitrap and time of flight (TOF) analyzers. TOF based instruments can achieve mass resolution up to 60,000 (at m/z 200), while Orbitrap based instruments can achieve much higher mass resolution—up to 240,000, and over 1,000,000 for FT-ICR (at m/z 400) [20]. HRMS provide several advantages in metabolomics and lipidomics studies, including high resolving power, increased mass accuracy and increased limits of detection [13]. High resolution and mass accuracy also allows for adduct identification with high precision [13]. Application of HRMS based DNA adductomics describing screening of known and unknown adducts of the DNA by using Orbitrap based multiple-stage mass spectrometry (MS^n, n = tandem stage) experiments was recently reported [23,24]. Due to these advantages, HRMS techniques are being increasingly used in metabolomics. Below, we summarize major advantages of FT-ICR and Orbitrap based instruments for metabolomics and lipidomics studies by HRMS:

(a) High mass resolution with the ability to achieve measurements with ppm and sub-ppm errors allows a complex metabolic extract to be analyzed with minimal chance of interference from overlap of other species in the mass spectrum [13]. The ability to discriminate metabolites at the 1–3 ppm level not only dramatically improves characterization of complex mixtures but also minimizes ambiguity of molecular formula assignments.
(b) Extremely high mass accuracy and sufficiently high acquisition rates makes FT-ICR and Orbitrap based instruments very popular in direct infusion mass spectrometry (DIMS), especially for metabolic and lipidomics fingerprinting studies. The ability of direct sample infusion would be clearly advantageous over existing time-consuming metabolite analyses or screening methods. With only a few minutes required for data acquisition with very high information content [12], DIMS can decrease the demand for extensive chromatographic separation and dramatically increase sample throughput in large scale screening experiments [12,25,26]. The high-throughput approach permits a sample to be processed within a few minutes and the short analysis time increases inter-sample reproducibility and improves the accuracy of subsequent cluster analysis [27].

(c) Flexibility in choosing the ion source or fragmentation technique. Variety of ion sources, including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), vacuum or atmospheric, matrix assisted laser desorption ionization (MALDI), desorption ionization (DESI), and direct analysis in real time (DART), are currently available and have been used for metabolomics and lipidomics applications [5,6,13,20,28,29]. Different fragmentation techniques like collision induced dissociation (CID), higher-energy collisional dissociation (HCD), electron induced dissociation (EID), infrared multiphoton dissociation (IRMPD), electron-transfer dissociation (ETD) and electron-transfer and higher-energy collision dissociation (EThcD) are available at any stage of MS\textsuperscript{n} with detection in either the Orbitrap or linear ion trap detector [20].

(d) Ability to perform stepwise fragmentation in multiple stage mass spectrometry (MS\textsuperscript{n}) experiments is useful for more confident metabolite identification or in-depth metabolite characterization of unknown compounds [7].

3. HRMS Applications in Metabolomics and Lipidomics

Numerous studies have been published employing HRMS based techniques in metabolomics [30–41], lipidomics [2,22,42–67], glycomics [68–70], food chemistry [71–73], natural products discovery [74,75], environmental [76,77] and pharmaceutical [78,79] studies.

HRMS based metabolomics and lipidomics can be performed either by a shotgun approach based on DIMS where samples are directly infused into a mass spectrometer or using chromatographic or electrophoretic separation prior to MS detection.

3.1. Shotgun Based Approaches

Shotgun approaches based on DIMS are being widely used due to their simplicity, limited sample prep and high throughput. Additionally, the data from direct infusion experiments can be directly used in multivariate statistical analysis without complicated data pre-processing steps. DIMS was successfully applied to metabolomics and lipidomics studies using both FT-ICR and Orbitrap analyzers.

DIMS provides several advantages to large scale metabolomics studies where analysis speed and sample throughput is most important. High mass accuracy and resolution of FT-ICR and Orbitrap instruments can significantly increase the number of molecular species detected in fingerprinting experiments. Even though many metabolites can be observed in DIMS, the majority of the ions remain unidentified. Therefore, this approach is often used in metabolic or lipidomic fingerprinting experiments [80].

Aharoni and co-authors [12] used a direct infusion FTMS approach for high throughput metabolic screening of differentially expressed metabolites in a mutant strawberry population with a relative quantitation and putative identification. Since different isomers sometimes show identical empirical formulas, the data obtained by FTMS experiment was further correlated with data obtained from gene expression studies using DNA microarrays [12]. Authors also suggested the use of preferably similar matrices to avoid any ion suppression and elimination of the adduct formation. In another study, Witting and co-authors [31] used direct-infusion ion-cyclotron-resonance Fourier-transform mass spectrometry (DI-ICR-FT-MS) in non-targeted metabolomics to obtain high-resolution snapshots
of the metabolic state of a *Caenorhabditis elegans* interacting with pathogens. They identified marked decrease in amino-acid metabolism with infection by *Pseudomonas aeruginosa* and a marked increase in sugar metabolism with infection by *Salmonella enterica*.

The shotgun approach, in the case of lipidomics, is proven to be an effective method to get quick snapshots of molecular composition of complex lipidomes, and this approach has been demonstrated successfully in combination with HRMS [2,22,42,49,51,58,59,62,81–86]. The Orbitrap mass spectrometers are especially useful in shotgun lipidomics because of their rapid acquisition of MS/MS spectra, higher mass resolution and optional MS^n^ fragmentation [2,42,44], and, most importantly, the rapid polarity switching with sub-ppm mass accuracy, which ultimately simplifies and accelerates the shotgun lipidomics analysis and improves lipidome coverage [42].

Matrix assisted laser desorption ionization (MALDI) coupled to FTMS is another approach that can be used for shotgun analysis. This technique, for example, was used by Wang and co-authors to study urinary metabolites, mainly focused on prediction of acute cellular renal allograft rejection [87] and acute tubular injury [88] through urinary metabolomics. Both studies suggest that the use of MALDI resulted in production of singly charged species and higher sensitivity and specificity [87,88].

Despite many advantages, D IMS and other shotgun approaches suffer significant drawbacks mostly related to their limited ability to resolve isobaric species or co-suppression effect where useful signals from many metabolites are lost at the mass spectrometer interface. To minimize co-suppression effect, two-step fingerprinting/validating strategy [89] or fractionated fingerprinting approach [90,91] can be used for metabolic fingerprinting.

### 3.2. Hyphenated Techniques

To overcome issues related to sample complexity, co-suppression, and improve resolution of isobaric species, FTMS is often used in combination with front-end chromatography or electrophoresis separation techniques like gas chromatography (GC), liquid chromatography (LC), ion chromatography (IC) or capillary electrophoresis (CE) (reviewed by [21,92]).

LC-MS analysis has been extensively used in metabolomics and lipidomics studies over the last decade [93–118]. It offers high sensitivity, high resolution and covers wide polarity and molecular weight range of analytes. Over the years, a large number of LC-MS based techniques have been developed to study many metabolite classes.

Recent advances in LC separation methodology, including development of ultra-performance liquid chromatography (UPLC), using capillary monolithic columns, and application of fused core particles, significantly improved chromatographic resolution and resulted in increased analysis speed and metabolite coverage, which is critical in large scale metabolomics experiments.

Application of solid or fused-core particles can provide faster chromatographic separation and increased sample throughput. Hu and co-authors [93] demonstrated the development and validation of the LC-FT-ICR-MS method for profiling of lipids in human and mouse plasma using a fused-core column. They used a C8 column with 2.7 µm fused-core silica particles and a 0.5 µm thick porous shell, which allows higher flow rates and faster separations that are subsequently detected by FTMS [93].

In a recent study, Granafei and co-authors [119] demonstrated the use of fused-core ultrapure silica particles (2.7 µm) narrow bore column in combination with LC-ESI-FTMS for the identification of isobaric lyso-phosphatidylcholines (LPC) in lipid extract of gilthead sea bream, which led to significant improvement in chromatographic resolution of phospholipids and, in combination with Orbitrap MS, it was useful to resolve the remarkable complexity of LPC [119]. Solid or fused core particles are now available in many different phases and particle sizes (from 1.6 to 5 µm) and are provided by multiple commercial vendors. Damen and co-authors described a novel approach for the separation of different lipid molecular species and lipid isomers in human plasma using a stationary phase incorporating charged surface hybrid (CSH) technology using reversed-phase UPLC combined with ion-mobility and HRMS [120].
To increase metabolite coverage, it is plausible to use multiple chromatographic separations utilizing different column chemistry and combine data from these separations. Soltow and co-authors, for example, used a dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) approach to increase the number of detected metabolites in their study of the exposome [26]. Authors performed sequential LC-FTMS analyses using reverse phase (C18) chromatography and anion exchange (AE) chromatography. This approach increased m/z feature detection by 23%–36%, yielding a total number of features up to 7000 for individual samples when compared to analysis with the AE column alone. From all detected features, approximately 50% of the m/z was matched to known chemicals in metabolomic databases, and 23% of the m/z were common to analyses on both columns.

Significant technical advances for the HRMS-based metabolomics in the past few years was the introduction of the GC-enabled quadrupole linear ion trap (QLT)-Orbitrap hybrid mass spectrometer capable of high resolution (up to 100,000 at m/z 400) and sub-parts-per-million mass accuracy GC-MS [121]. The performance of the new instrument was demonstrated by its application to the determination of polychlorinated dibenzo-p-dioxins and dibenzofuran in the environmental samples and profiling of primary metabolites in *Arabidopsis thaliana* extracts [121]. Later, Peterson and co-authors [18,19] reported the development of GC/Quadrupole Orbitrap mass spectrometer which combines high mass accuracy, high resolution, and high sensitivity analyte detection that makes it a promising instrument for both untargeted and targeted metabolomics studies. The authors also developed an “intelligent” data-dependent algorithm, termed molecular ion directed acquisition (MIDA). This algorithm maximizes the information content generated from unsupervised tandem MS and selected ion monitoring (SIM) by directing the MS to target the ions of greatest information content [18]. New instruments and software were successfully used for non-targeted metabolomics. Combination of $^{13}$C- and $^{15}$N-metabolic labeling, multiple derivatization and ionization types, and heuristic filtering of candidate elemental compositions allowed to achieve MS/MS spectra of nearly all intact ion species for structural elucidation, knowledge of carbon and nitrogen atom content for every ion in MS and MS/MS spectra, relative quantification between alternatively labeled samples, and unambiguous annotation of elemental composition [18,19]. This proved it to be a very promising technology in discovery metabolomics to study volatile compounds or compounds that can be volatilized by chemical derivatization.

### 3.3. Mass Spectrometry Imaging

One of the drawbacks of the DIMS and most hyphenated techniques is their inability to provide spatial information on localization of various metabolites and lipids in organs and tissues. The mass spectrometry imaging (MSI) approach can provide this spatial information. Multiple MSI approaches based on different ionization techniques have been developed and are currently being widely used. Among them, MALDI combined with HRMS is the most often used in imaging applications. It has been successfully used for imaging a variety of human [122–126], animal [127–131] and plant [132,133] tissues. For example, spatial mapping of lipids at cellular resolution in cotton embryos using MALDI Hybrid Ion Trap-Orbitrap (MALDI LTQ Orbitrap XL) mass spectrometer showed differential distribution of lipid species such as triglycerols and phosphatidylcholines [133,134]. Many other lipidomics applications of MALDI imaging were subsequently reviewed by [133–135]. Most MSI applications are currently focused on imaging lipids [136–143], although imaging of primary and specialized metabolites and xenobiotics has been reported [122,128,132,144–146].

Ambient ionization methods, such as atmospheric pressure MALDI (AP-MALDI) [147–151], desorption electrospray ionization (DESI) [152,153] and matrix-assisted laser desorption electrospray ionization (MALDESI) [146,154–156], have also been employed for MSI. Ambient ionization approaches can provide certain advantages over the vacuum MALDI source for MSI. For example, AP-MALDI can provide high spatial resolution (below 10 μm) with high mass resolution and high mass accuracy obtained with Orbitrap-based instrumentation [147–151].
3.4. Other Applications of HRMS

Analytical advantages of HRMS make it broadly applicable to other fields beyond metabolomics and lipidomics. Numerous studies have been published employing HRMS based techniques in many research areas, such as glycomics [68–70], food science [71–73], forensics [157], toxicology [157,158], natural products discovery [74,75], agriculture [37,96,159–167], environmental [76,77] and pharmaceutical [78,79] studies. In recent years, food authenticity and safety have become a global concern prompting the development of novel analytical techniques to address food safety issues. The role of HRMS in many studies has proven it to be crucial for understanding process contamination, food adulteration and food contaminants, such as pesticides and mycotoxins [71,72,159–162]. In a case study of doping control, Kiss and colleagues used ultra HRMS based non-targeted metabolomics to study salbutamol and budesonide abuse through analysis of human urinary metabolites [168]. In another doping control study, markers of testosterone misuse were analyzed by untargeted metabolomics approach and HRMS [79]. Applications of HRMS in agriculture range from determining mycotoxins in agricultural products [163–165] and profiling human health related metabolites in crop plants [169–171] to studying the effect of different diets on animal metabolism [37]. In recent study, Sun and co-authors studied the effect of high fat, high cholesterol diet on changes in metabolite patterns in pigs [37]. They analyzed plasma, fecal and urine samples from pigs fed high fat or basal regular diets using Ultra High Performance Liquid Chromatography (UHPLC)-HRMS and chemometric analysis and found a set of metabolites most affected by the diet [37,172]. Although the application of metabolomics in environmental studies for the analysis of environmental pollutants has been reported [164,167,173–179], the use of HRMS based MS techniques for environmental research is still limited [76,77]. Applications of mass spectrometry in the pharmaceutical metabolomics can be further expanded by using HRMS (reviewed by Drexler and colleagues [78]).

4. Data Analysis and Databases

Metabolomics experiments based on non-targeted HRMS analysis generate large amounts of data and require extensive raw data pre-processing and application of specialized mathematical, statistical and bioinformatics tools [92,180–183]. Pre-processing can be done by using in-house or specialized tools [184,185]. Multivariate statistics tools commonly used to analyze metabolomics data include pattern recognition, identification of outliers, reduction of data dimensionality, and compression of large datasets [92,182,186].

As FTMS provides the resolution and mass accuracy that are required to reliably assign molecular formulas to detected ions, it is imperative to use this information to metabolite identifications. It is generally accepted that accurate mass alone is not sufficient to positively identify an unknown structure, and the chemical structure database returns multiple hits (sometime several hundred or more) at a defined mass tolerance window. Even at the highest mass resolution, FTMS cannot provide exact identification because many isobaric species and structural isomers have identical empirical formulas. In such cases, additional information, including chromatographic retention time, isotope pattern matching, collisional cross section (CCS), and use of multiple stage mass spectrometry (MS\(^n\)) is needed for correct compound annotation [187–189]. MS data can also be correlated with nuclear magnetic resonance (NMR) data.

Numerous chemical reference databases and mass spectral libraries are currently publicly available. There is also a significant increase in accurate mass enabled mass spectral databases in recent years. Reference chemical and biochemical databases are either focused on collecting reference information on chemical compounds independently of their sources or provide information on endogenous and exogenous metabolites linked to a particular biological system or matrix. Some databases are limited to a particular metabolite classes. Among the largest chemical databases are PubChem [190–192] and ChemSpider [193–195]. Mass spectral libraries and databases containing fragmentation data are invaluable resources for compound identification. Until recently, many MS/MS databases contained only nominal mass spectral data. The increased application of HRMS
in metabolomics leads to the development of accurate mass enabled spectral search programs and databases that contain information on accurate mass of just the precursor or both the precursor and fragment ions. The popular NIST (National Institute of Standards and Technology) MS Search program version 2.0 released in 2011 (Standard Reference Data, NIST, Gaithersburg, MD, USA) allows for exact mass search of parent and fragment ion. Many open-access spectral databases also contain accurate mass information and high resolution mass spectra. For example, the Scripps Center for Metabolomics released the online database called METLIN [196] which is a repository of metabolite information and tandem mass spectrometry data designed to facilitate metabolite identification in metabolomics [33,197–199]. The database provides comprehensive MS/MS metabolite data and each metabolite is linked to outside resources like Kyoto Encyclopedia of Genes and Genomes (KEGG) for further reference. The webserver MassTRIX [200–202] provides assignment of the bulk chemical formulae considering biological and genomic context of the samples. The mass difference network based approach for formula calculation [203] or on data combination from lower resolution LC-MS with FT-ICR-MS [204] can also be used.

Other metabolomics databases used in many metabolomics studies include KEGG [205,206], Madison Metabolomics Consortium Database (MMCD) [207], Human Metabolome Database and drug bank [208,209], and LIPID Metabolites and Pathway Strategy (LIPIDMAPS) [210,211]. Tools for putative metabolite identification using multiple online databases, e.g., MetaboSearch, can simplify concurrent searches of multiple metabolite databases [212].

Specialized algorithms for profiling individual metabolite classes have also been developed [213,214]. For example, the program “LipidSearch” developed by Taguchi and co-authors [213], utilizes specific detection approach by neutral loss survey-dependent MS3, for the identification of molecular species of phosphatidylcholine, sphingomyelin and phosphatidylserine. LipidSearch program combined with HRMS was successfully used for lipid annotation in multiple studies [213,215–219]. Several other programs such as LipidQA [220], LIMSA [221], FAAc [222], lipID [223], LipidView [50], LipidInspector [46] (Herzog et al.) are specialized in identification of lipids from the shotgun experiments. Extensive review of bioinformatics tools and software can be found in the literature [44,224]. A novel approach to represent and calculate the similarity between high-resolution mass spectral trees (Figure 1) has been proposed for the construction of the MS3 libraries for the annotation and structural elucidation of the unknown metabolites. Structures of the unknown metabolites can be predicted in a high throughput approach utilizing fragmentation trees and precursor ion fingerprinting (PIF) technique [7,225,226]. This approach utilizes structural information from high resolution fragmentation spectra and predicts the identity of the unknown metabolite by determining its possible substructures (Figure 1). High resolution MS3 spectra of unknown metabolites are first collected using the spectral ion tree approach. The product ion MS3 spectra of various precursor ions are then searched against the MS3 spectral library. Although the unknown metabolite is not present in the spectral library, the spectra of molecules that contain similar substructures will provide unambiguous substructure identifications. Identified substructures/fragments are used to generate a structural proposal for unknown metabolite (Figure 2).

Until recently, wider use of this approach was limited by the lack of publically available curated spectral tree databases. The newly developed mzCloud library [227] provides a significant step forward in this direction. mzCloud is a novel type of mass spectral database that can help to predict structures of unknown metabolites and identify compounds even when they are not present in the mass spectral library using PIF technique [226]. This is of immense value when traditional library search yields no results. PIF relies on well defined and chemically plausible structures of fragment ions, which are either used to reassemble the parent compound or, at the very least, point towards its structural characteristics. The library contains substructurally characterized precursor ions of MS3 spectra calculated using heuristic and quantum chemical methods. The quantum chemical annotation pipeline for precursor ion prediction contains over 500,000 unique 3D structures with calculated thermochemical properties in semi-empirical and Discrete Fourier Transform (DFT) levels of theory. Each spectral peak present in the library is annotated by one or more alternative molecular formulas.
that can be displayed in the spectra. mzCloud employs a freely searchable collection of manually curated, high resolution/accurate mass spectra based on the cloud technology. mzCloud library has an advanced database viewer which displays: spectral trees, MS\textsuperscript{n} spectra, structures, fragments, fragmentation patterns, collision energies, resolution, accuracy, isolation width, names, break-down curves and other relevant information. To date, the mzCloud database features over 1,00,000 processed spectral records covering a wide range of collision energies up to MS\textsuperscript{8} in 4300 endogenous metabolites, plant secondary metabolites, food additives, pharmaceuticals, environmental contaminants and other compounds relevant for metabolomics.

**Figure 1.** In tandem mass spectrometry, a spectral tree is a data structure encapsulating the hierarchically organized product ion mass spectra of a single chemical compound where each level represents an MS\textsuperscript{n} stage. Edges refer to precursor m/z values, and nodes refer to spectra generated from the particular precursor ions.

**Figure 2.** Strategy for structure elucidation for unknown metabolites using mzCloud library of mass spectral fragments/substructures.
5. Conclusions: Technological Developments and Future Perspectives

FTMS and Orbitrap based MS technology have proven to be useful for untargeted or targeted screening and a broad range of qualitative and quantitative applications in diverse fields like metabolomics, lipidomics, drug discovery, proteomics, environmental and food safety, clinical research, forensic toxicology and agricultural science. Today, we have some of the best and highly advanced FTMS and Orbitrap systems available on the market. Modern FTMS systems offer ultra-high mass resolution of 10,000,000, while current Orbitrap systems offer less than 1 ppm mass accuracy, up to 450,000 FWHM, more than four orders of magnitude intrascan dynamic range, along with femtogram-level sensitivity, a fast scanning rate at 15 Hz and spectral multiplexing suited to UHPLC applications and mass range to 6000 Da. Improvement in instrumentation was accompanied by development of new data processing algorithms, software and databases, many of which are available in the public domain.

In the near future, we should see more improvements in instrumentation and processing software, specifically in increasing data acquisition rate, improving isotope ratio accuracy, exploring more hyphenated techniques and multi-dimensional chromatography, and creating integrated and flexible data processing solutions from raw data to biological interpretation. It is also necessary to increase community efforts in standardizing metabolomics and lipidomics data and metadata standards, and expanding bioinformatics tools and metabolomics data repositories available to the community.

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Abbreviations
The following abbreviations are used in this manuscript:

- AE Anion exchange
- APCI Atmospheric pressure chemical ionization
- AP-MALDI Atmospheric pressure matrix-assisted laser desorption ionization
- ATI Acute tubular injury
- CCS Collisional cross section
- CE-MS Capillary electrophoresis mass spectrometry
- CID Collision induced dissociation
- CSH Charged surface hybrid
- DART Direct analysis in real time
- DC-FTMS Dual chromatography-Fourier transform mass spectrometry
- DESI Desorption electrospray ionization
- DFT Discrete Fourier transform
- DIMS Direct infusion mass spectrometry
- DNA Deoxyribonucleic acid
- EID Electron induced dissociation
- ESI Electro spray ionization
- ETD Electron-transfer dissociation
- EThcD Electron-transfer and higher-energy collision dissociation
- FT-ICR-MS Fourier transform ion cyclotron resonance mass spectrometry
- FTMS Fourier transform mass spectrometry
- FWHM Full width at half maximum
- GC-MS Gas chromatography coupled to mass spectrometry
- HCD Higher-energy collisional dissociation
- HRMS High resolution mass spectrometry
- IC Ion chromatography
- IRMPD Infrared multiphoton dissociation
- KEGG Kyoto encyclopedia of genes and genomes
- LC-FT-ICR-MS Liquid chromatography-Fourier transform ion cyclotron resonance mass spectrometry
LC-FTMS | Liquid chromatography-Fourier transform mass spectrometry
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LC-MS | Liquid chromatography coupled to mass spectrometry
LPC | Lyso-phosphatidylcholines
m/z | Mass to charge ratio
MALDESI | Matrix-assisted laser desorption electro spray ionization
MALDI | Matrix assisted laser desorption ionization
MIDA | Molecular ion directed acquisition
MMCD | Madison metabolomics consortium database
MS/MS | Tandem mass spectrometry
MS | Mass spectrometry
MSI | Mass spectrometry imaging
MS^n | Multiple-stage mass spectrometry
NMR | Nuclear magnetic resonance
PCDD | Polychlorinated dibenzo-p-dioxins
PCDF | Polychlorinated dibenzofurans
PIF | Precursor ion fingerprinting
SIM | Selected ion monitoring
UHPLC | Ultra high performance liquid chromatography
UPLC | Ultra performance liquid chromatography

References
1. Oliver, S.G.; Winson, M.K.; Kell, D.B.; Baganz, F. Systematic functional analysis of the yeast genome. Trends Biotechnol. 1998, 16, 373–378. [CrossRef]
2. Schuhmann, K.; Herzog, R.; Schwudke, D.; Metelmann-Strupat, W.; Bornstein, S.R.; Shevchenko, A. Bottom-up shotgun lipidomics by higher energy collisional dissociation on LTQ Orbitrap mass spectrometers. Anal. Chem. 2011, 83, 5480–5487. [CrossRef] [PubMed]
3. Soltow, Q.A.; Jones, D.P.; Promislow, D.E. A network perspective on metabolism and aging. Integr. Comp. Biol. 2010, 50, 844–854. [CrossRef] [PubMed]
4. Want, E.J.; Nordstrom, A.; Morita, H.; Siuzdak, G. From exogenous to endogenous: The inevitable imprint of mass spectrometry in metabolomics. J. Proteome Res. 2007, 6, 459–468. [CrossRef] [PubMed]
5. Kuehnbaum, N.L.; Britz-McKibbin, P. New advances in separation science for metabolomics: Resolving chemical diversity in a post-genomic era. Chem. Rev. 2013, 113, 2437–2468. [CrossRef] [PubMed]
6. Nagornov, K.O.; Gorshkov, M.V.; Kozhinov, A.N.; Tsybin, Y.O. High-resolution Fourier transform ion cyclotron resonance mass spectrometry with increased throughput for biomolecular analysis. Anal. Chem. 2014, 86, 9020–9028. [CrossRef] [PubMed]
7. Rojas-Cherto, M.; Peironcely, J.E.; Kasper, P.T.; van der Hooft, J.J.; de Vos, R.C.; Vreeken, R.; Hankemeier, T.; Reijmers, T. Metabolite identification using automated comparison of high-resolution multistage mass spectral trees. Anal. Chem. 2012, 84, 5524–5534. [CrossRef] [PubMed]
8. Erve, J.C.; Demaio, W.; Talaat, R.E. Rapid metabolite identification with sub parts-per-million mass accuracy from biological matrices by direct infusion nanoelectrospray ionization after clean-up on a ZipTip and LTQ/Orbitrap mass spectrometry. Rapid Commun. Mass Spectrom. 2008, 22, 3015–3026. [CrossRef] [PubMed]
9. Comisarow, M.B.; Marshall, A.G. The early development of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. J. Mass Spectrom. 1996, 31, 581–585. [CrossRef]
10. Marshall, A.G.; Hendrickson, C.L.; Jackson, G.S. Fourier transform ion cyclotron resonance mass spectrometry: A primer. Mass Spectrom. Rev. 1998, 17, 1–35. [CrossRef]
11. Villas-Boas, S.G.; Mas, S.; Akesson, M.; Smidsraaard, J.; Nielsen, J. Mass spectrometry in metabolome analysis. Mass Spectrom. Rev. 2005, 24, 613–646. [CrossRef] [PubMed]
12. Aharoni, A.; Ric de Vos, C.H.; Verhoeven, H.A.; Maliepaard, C.A.; Kruppa, G.; Bino, R.; Goedenowe, D.B. Nontargeted metabolome analysis by use of Fourier Transform Ion Cyclotron Mass Spectrometry. Omics J. Integr. Biol. 2002, 6, 217–234. [CrossRef] [PubMed]
13. Brown, S.C.; Kruppa, G.; Dasseux, J.L. Metabolomics applications of FT-ICR mass spectrometry. Mass Spectrom. Rev. 2005, 24, 223–231. [CrossRef] [PubMed]
14. Jeandet, P.; Heinzmann, S.S.; Rouiller-Gall, C.; Gilindre, C.; Aron, A.; Deville, M.A.; Moritz, F.; Karbowiak, T.; Demarville, D.; Brun, C.; et al. Chemical messages in 170-year-old champagne bottles from the Baltic Sea: Revealing tastes from the past. Proc. Natl. Acad. Sci. USA 2015, 112, 5893–5898. [CrossRef] [PubMed]
15. Comisarow, M.B.; Marshall, A.G. Fourier transform ion cyclotron resonance spectroscopy. *Chem. Phys. Lett.* 1974, 25, 282–283. [CrossRef]

16. Hu, Q.; Noll, R.J.; Li, H.; Makarow, A.; Hardman, M.; Graham Cooks, R. The Orbitrap: A new mass spectrometer. *J. Mass Spectrom.* 2005, 40, 430–443. [CrossRef] [PubMed]

17. Makarow, A.; Scigelova, M. Coupling liquid chromatography to Orbitrap mass spectrometry. *J. Chromatogr. A* 2010, 1217, 3938–3945. [CrossRef] [PubMed]

18. Peterson, A.C.; Balloon, A.J.; Westphall, M.S.; Coon, J.J. Development of a GC/Quadrupole-Orbitrap mass spectrometer, part II: New approaches for discovery metabolomics. *Anal. Chem.* 2014, 86, 10044–10051. [CrossRef] [PubMed]

19. Peterson, A.C.; Hauschild, J.P.; Quarmby, S.T.; Krumwiede, D.; Lange, O.; Lemke, R.A.; Grosse-Coosmann, F.; Horning, S.; Donohue, T.J.; Westphall, M.S.; et al. Development of a GC/Quadrupole-Orbitrap mass spectrometer, part I: Design and characterization. *Anal. Chem.* 2014, 86, 10036–10043. [CrossRef] [PubMed]

20. Junot, C.; Fenaille, F.; Colsch, B.; Becher, F. High resolution mass spectrometry based techniques at the crossroads of metabolomic pathways. *Mass Spectrom. Rev.* 2014, 33, 471–500. [CrossRef] [PubMed]

21. Junot, C.; Madalinski, G.; Tabet, J.C.; Ezan, E. Fourier transform mass spectrometry for metabolome analysis. *Analyst* 2010, 135, 2203–2219. [CrossRef] [PubMed]

22. Schwudke, D.; Schuhmann, K.; Herzog, R.; Bornstein, S.R.; Shevchenko, A. Shotgun lipidomics on high resolution mass spectrometers. *Cold Spring Harbor Perspect. Biol.* 2011, 3, a004614. [CrossRef] [PubMed]

23. Balbo, S.; Turesky, R.J.; Villalta, P.W. DNA adductomics. *Chem. Res. Toxicol.* 2014, 27, 356–366. [CrossRef] [PubMed]

24. Balbo, S.; Hecht, S.S.; Upadhyaya, P.; Villalta, P.W. Application of a high-resolution mass-spectrometry-based DNA adductomics approach for identification of DNA adducts in complex mixtures. *Anal. Chem.* 2014, 86, 1744–1752. [CrossRef] [PubMed]

25. Raterink, R.-J.; Kloet, F.M.; Li, J.; Wattel, N.A.; Schaaf, M.J.M.; Spaink, H.P.; Berger, R.; Vreeken, R.J.; Hankemeier, T. Rapid metabolic screening of early zebrafish embryogenesis based on direct infusion-nanoESI-FTMS. *Metabolomics* 2013, 9, 864–873. [CrossRef]

26. Soltow, Q.A.; Strobel, F.H.; Mansfield, K.G.; Wachtman, L.; Park, Y.; Jones, D.P. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome. *Metabolomics* 2013, 9, S132–S143. [CrossRef] [PubMed]

27. Dettmer, K.; Aronov, P.A.; Hammock, B.D. Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.* 2007, 26, 51–78. [CrossRef] [PubMed]

28. Lei, Z.; Huhman, D.V.; Sumner, L.W. Mass spectrometry strategies in metabolomics. *J. Biol. Chem.* 2011, 286, 25435–25442. [CrossRef] [PubMed]

29. Moco, S.; Capanoglu, E.; Tikunov, Y.; Bino, R.J.; Boyacioglu, D.; Hall, R.D.; Vervoort, J.; de Vos, R.C. Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *J. Exp. Bot.* 2007, 58, 4131–4146. [CrossRef] [PubMed]

30. Wishart, D.S.; Lewis, M.J.; Morrissey, J.A.; Flegel, M.D.; Jeroncic, K.; Xiong, Y.; Cheng, D.; Eisner, R.; Gautam, B.; Tzur, D.; et al. The human cerebrospinal fluid metabolome. *J. Chromatogr. B* 2008, 871, 164–173. [CrossRef] [PubMed]

31. Witting, M.; Lucio, M.; Tziotis, D.; Wagele, B.; Suhre, K.; Voulhoux, R.; Garvis, S.; Schmitt-Kopplin, P. DI-ICR-FT-MS-based high-throughput deep metabotyping: A case study of the *Caenorhabditis elegans-Pseudomonas aeruginosa* infection model. *Anal. Bioanal. Chem.* 2015, 407, 1059–1073. [CrossRef] [PubMed]

32. Zhou, J.; Weber, R.J.; Allwood, J.W.; Mistrik, R.; Zhu, Z.; Ji, Z.; Chen, S.; Dunn, W.B.; He, S.; Viant, M.R. HAMMER: Automated operation of mass frontier to construct in silico mass spectral fragmentation libraries. *Bioinformatics* 2014, 30, 581–583. [CrossRef] [PubMed]

33. Zhu, Z.J.; Schultz, A.W.; Wang, J.; Johnson, C.H.; Yannone, S.M.; Patti, G.J.; Siuzdak, G. Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. *Nat. Protoc.* 2013, 8, 451–460. [CrossRef] [PubMed]

34. Meijon, M.; Feito, I.; Oravec, M.; Delatorre, C.; Weckwerth, W.; Majada, J.; Valledor, L. Exploring natural variation of *Pinus pinaster* Aiton using metabolomics: Is it possible to identify the region of origin of a pine from its metabolites? *Mol. Ecol.* 2016, 25, 959–976. [CrossRef] [PubMed]
35. Diaz, R.; Gallart-Ayala, H.; Sancho, J.V.; Nunez, O.; Zamora, T.; Martins, C.P.; Hernandez, F.; Hernandez-Cassou, S.; Saurina, J.; Checa, A. Told through the wine: A liquid chromatography-mass spectrometry interplatform comparison reveals the influence of the global approach on the final annotated metabolites in non-targeted metabolomics. *J. Chromatogr. A* 2016, 1433, 90–97. [CrossRef] [PubMed]

36. Thevenot, E.A.; Roux, A.; Xu, Y.; Ezan, E.; Junot, C. Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *J. Proteome Res.* 2015, 14, 3322–3335. [CrossRef] [PubMed]

37. Sun, J.; Monagas, M.; Jang, S.; Molokin, A.; Harñyl, J.M.; Urban, J.F., Jr.; Solano-Aguilar, G.; Chen, P. A high fat, high cholesterol diet leads to changes in metabolite patterns in pigs—A metabolomic study. *Food Chem.* 2015, 173, 171–178. [CrossRef] [PubMed]

38. Sun, J.; Kou, L.; Geng, P.; Huang, H.; Yang, T.; Luo, Y.; Chen, P. Metabolomic assessment reveals an elevated level of glucosinolate content in CaCl$_2$ treated broccoli microgreens. *J. Agric. Food Chem.* 2015, 63, 1863–1868. [CrossRef] [PubMed]

39. Shen, C.; Sun, Z.; Chen, D.; Su, X.; Jiang, J.; Li, G.; Lin, B.; Yan, J. Developing urinary metabolic signatures as early bladder cancer diagnostic markers. *Omics J. Integr. Biol.* 2015, 19, 1–11. [CrossRef] [PubMed]

40. Scalabrin, E.; Radaelli, M.; Rizzato, G.; Bogani, P.; Buiaiti, M.; Gambaro, A.; Capodaglio, G. Metabolomic analysis of wild and transgenic Nicotiana langsdorffii plants exposed to abiotic stresses: Unraveling metabolic responses. *Anal. Bioanal. Chem.* 2015, 407, 6357–6368. [CrossRef] [PubMed]

41. Nicolardi, S.; Bogdanov, B.; Deelder, A.M.; Palmblad, M.; van der Burgt, Y.E. Developments in FTICR-MS and its potential for body fluid signatures. *Int. J. Mol. Sci.* 2015, 16, 27133–27144. [CrossRef] [PubMed]

42. Schuhmann, K.; Almeida, R.; Baumert, M.; Herzog, R.; Bornstein, S.R.; Shevchenko, A. Shotgun lipidomics on a LTQ Orbitrap mass spectrometer by successive switching between acquisition polarity modes. *J. Mass Spectrom.* 2012, 47, 96–104. [CrossRef] [PubMed]

43. Kouman, A.; Woffendin, G.; Narayana, V.K.; Welchman, H.; Crone, C.; Volmer, D.A. High-resolution extracted ion chromatography, a new tool for metabolomics and lipidomics using a second-generation orbitrap mass spectrometer. *Rapid Commun. Mass Spectrom.* 2009, 23, 1411–1418. [CrossRef] [PubMed]

44. Herzog, R.; Schwudke, D.; Schuhmann, K.; Sampaio, J.L.; Bornstein, S.R.; Schroeder, M.; Shevchenko, A. A novel informatics concept for high-throughput shotgun lipidomics based on the molecular fragmentation query language. *Genome Biol.* 2011, 12, R8. [CrossRef] [PubMed]

45. Graessler, J.; Schwudke, D.; Schwarz, P.E.; Herzog, R.; Shevchenko, A.; Bornstein, S.R. Top-down lipidomics reveals ether lipid deficiency in blood plasma of hypertensive patients. *PLoS ONE* 2009, 4, e6261. [CrossRef] [PubMed]

46. Schwudke, D.; Liebisch, G.; Herzog, R.; Schmitz, G.; Shevchenko, A. Shotgun lipidomics by tandem mass spectrometry under data-dependent acquisition control. *Methods Enzymol.* 2007, 433, 175–191. [PubMed]

47. Shevchenko, A.; Simons, K. Lipidomics: Coming to grips with lipid diversity. *Nat. Rev. Mol. Cell Biol.* 2010, 11, 593–598. [CrossRef] [PubMed]

48. Matyash, V.; Liebisch, G.; Kurzchalia, T.V.; Shevchenko, A.; Schwudke, D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J. Lipid Res.* 2008, 49, 1137–1146. [CrossRef] [PubMed]

49. Almeida, R.; Pauling, J.K.; Sokol, E.; Hannibal-Bach, H.K.; Ejsing, C.S. Comprehensive lipidome analysis by shotgun lipidomics on a hybrid quadrupole-orbitrap-linear ion trap mass spectrometer. *J. Am. Soc. Mass Spectrom.* 2015, 26, 133–148. [CrossRef] [PubMed]

50. Ejsing, C.S.; Duchoslav, E.; Sampaio, J.; Simons, K.; Bonner, R.; Thiele, C.; Ekroos, K.; Shevchenko, A. Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. *Anal. Chem.* 2006, 78, 6202–6214. [CrossRef] [PubMed]

51. Tarasov, K.; Stefanko, A.; Casanovas, A.; Surma, M.A.; Berzina, Z.; Hannibal-Bach, H.K.; Ekroos, K.; Ejsing, C.S. High-content screening of yeast mutant libraries by shotgun lipidomics. *Mol. Biosyst.* 2014, 10, 1364–1376. [CrossRef] [PubMed]

52. Almeida, R.; Berzina, Z.; Arnspan, E.C.; Baumgart, J.; Vogt, J.; Nitsch, R.; Ejsing, C.S. Quantitative spatial analysis of the mouse brain lipidome by pressurized liquid extraction surface analysis. *Anal. Chem.* 2015, 87, 1749–1756. [CrossRef] [PubMed]

53. Casanovas, A.; Hannibal-Bach, H.K.; Jensen, O.N.; Ejsing, C.S. Shotgun lipidomic analysis of chemically sulfated sterols compromises analytical sensitivity: Recommendation for large-scale global lipidome analysis. *Eur. J. Lipid Sci. Technol.* 2014, 116, 1618–1620. [CrossRef] [PubMed]
54. Hansen, M.L.; Clausen, A.; Ejsing, C.S.; Risbo, J. Modulation of the Lactobacillus acidophilus La-5 lipidome by different growth conditions. *Microbiology* 2015, 161, 1990–1998. [CrossRef] [PubMed]

55. Jensen, S.M.; Neesgaard, V.L.; Skjoldbjerg, S.L.; Brandl, M.; Ejsing, C.S.; Treusch, A.H. The effects of temperature and growth phase on the lipidomes of *Sulfolobus islandicus* and *Sulfolobus tokodaii*. *Life* 2015, 5, 1539–1566. [CrossRef] [PubMed]

56. Jensen, S.M.; Neesgaard, V.L.; Skjoldbjerg, S.L.; Brandl, M.; Ejsing, C.S.; Treusch, A.H. The effects of temperature and growth phase on the lipidomes of *Sulfolobus islandicus* and *Sulfolobus tokodaii*. *Life* 2015, 5, 1539–1566. [CrossRef] [PubMed]

57. Sokol, E.; Ulven, T.; Faergeman, N.J.; Ejsing, C.S. Comprehensive and quantitative profiling of lipid species in human milk, cow milk and a phospholipid-enriched milk formula by GC and MS/MS. *Eur. J. Lipid Sci. Technol.* 2015, 117, 751–759. [CrossRef] [PubMed]

58. Wang, M.; Wang, C.; Han, R.H.; Han, X. Novel advances in shotgun lipidomics for biology and medicine. *Prog. Lipid Res.* 2016, 61, 83–108. [CrossRef] [PubMed]

59. Wang, M.; Han, X. Advanced shotgun lipidomics for characterization of altered lipid patterns in neurodegenerative diseases and brain injury. *Methods Mol. Biol.* 2016, 1303, 405–422. [PubMed]

60. Hu, S.; Wang, J.; Ji, E.H.; Christison, T.; Lopez, L.; Huang, Y. Targeted metabolomic analysis of head and neck cancer cells using high performance ion chromatography coupled with a Q Exactive HF mass spectrometer. *Anal. Chem.* 2015, 87, 6371–6379. [CrossRef] [PubMed]

61. Bird, S.S.; Stavrovskaya, I.G.; Gathungu, R.M.; Tousi, F.; Kristal, B.S. Qualitative characterization of the rat liver mitochondrial lipidome using all ion fragmentation on an Exactive benchtop Orbitrap MS. *Methods Mol. Biol.* 2015, 1264, 441–452. [PubMed]

62. Wang, J.; Christison, T.T.; Misuno, K.; Lopez, L.; Hu, S. Metabolomic profiling of anionic metabolites in head and neck cancer cells by capillary ion chromatography with Orbitrap mass spectrometry. *Anal. Chem.* 2014, 86, 5116–5124. [CrossRef] [PubMed]

63. Zhang, R.; Watson, D.G.; Wang, L.; Westrop, G.D.; Coombs, G.H.; Zhang, T. Evaluation of mobile phase characteristics on three zwitterionic columns in hydrophilic interaction liquid chromatography mode for liquid chromatography-high resolution mass spectrometry based untargeted metabolite profiling of Leishmania parasites. *J. Chromatogr. A* 2014, 1362, 168–179. [CrossRef] [PubMed]

64. Wang, M.; Han, X. Multidimensional mass spectrometry-based shotgun lipidomics. *Methods Mol. Biol.* 2014, 1198, 203–220. [PubMed]

65. Wang, J.; Christison, T.T.; Misuno, K.; Lopez, L.; Huhmer, A.F.; Huang, Y.; Hu, S. Metabolomic profiling of anionic metabolites in head and neck cancer cells by capillary ion chromatography with Orbitrap mass spectrometry. *Anal. Chem.* 2014, 86, 5116–5124. [CrossRef] [PubMed]

66. Wang, M.; Fang, H.; Han, X. Shotgun lipidomics analysis of 4-hydroxyalkenal species directly from lipid extracts after one-step in situ derivatization. *Anal. Chem.* 2012, 84, 4580–4586. [CrossRef] [PubMed]

67. Senyuva, H.Z.; Gokmen, V.; Sarikaya, E.A. Future perspectives in Orbitrap™-high-resolution mass spectrometry in food analysis: A review. *Food Addit. Contam. Part A* 2015, 32, 1658–1606. [CrossRef] [PubMed]

68. Dalpathado, D.S.; Irungu, J.; Go, E.P.; Butnev, V.Y.; Norton, K.; Bousfield, G.R.; Desaire, H. Comparative glycomics of the glycoprotein follicle stimulating hormone: Glycopeptide analysis of isolates from two mammalian species. *Biochemistry* 2006, 45, 8665–8673. [CrossRef] [PubMed]

69. Senyuva, H.Z.; Gokmen, V.; Sarikaya, E.A. Future perspectives in Orbitrap™-high-resolution mass spectrometry in food analysis: A review. *Food Addit. Contam. Part A* 2015, 32, 1658–1606. [CrossRef] [PubMed]

70. Rubert, J.; Zachariasova, M.; Hajislova, J. Advances in high-resolution mass spectrometry based on metabolomics studies for food—A review. *Food Addit. Contam. Part A* 2015, 32, 1658–1708. [CrossRef] [PubMed]
73. Rizzuti, A.; Aguilera-Saez, L.M.; Gallo, V.; Cafagna, I.; Mastrorilli, P.; Latronico, M.; Pacifico, A.; Matarrese, A.M.; Ferrara, G. On the use of Ethephon as abscising agent in cv. Crimson Seedless table grape production: Combination of Fruit Detachment Force, Fruit Drop and metabolomics. *Food Chem.* 2015, 171, 341–350. [CrossRef] [PubMed]

74. Nielsen, K.F.; Larsen, T.O. The importance of mass spectrometric dereplication in fungal secondary metabolite analysis. *Front. Microbiol.* 2015, 6. [CrossRef] [PubMed]

75. Wolfender, J.L.; Marti, G.; Thomas, A.; Bertrand, S. Current approaches and challenges for the metabolite profiling of complex natural extracts. *J. Chromatogr. A* 2015, 1382, 136–164. [CrossRef] [PubMed]

76. Bundy, J.G.; Davey, M.P.; Viant, M.R. Environmental metabolomics: A critical review and future perspectives. *Metabolomics* 2008, 5, 3–21. [CrossRef]

77. Lankadurai, B.P.; Nagato, E.G.; Simpson, M.J. Environmental metabolomics: An emerging approach to study organism responses to environmental stressors. *Environ. Rev.* 2013, 21, 180–205. [CrossRef]

78. Drexler, D.M.; Reily, M.D.; Shipkova, P.A. Advances in mass spectrometry applied to pharmaceutical metabolomics. *Anal. Bioanal. Chem.* 2011, 399, 2645–2653. [CrossRef] [PubMed]

79. Raro, M.; Ibanez, M.; Gil, R.; Fabregat, A.; Tudela, E.; Deventer, K.; Ventura, R.; Segura, J.; Marcos, J.; Kotronoulas, A.; et al. Untargeted metabolomics in doping control: Detection of new markers of testosterone misuse by ultrahigh performance liquid chromatography coupled to high-resolution mass spectrometry. *Anal. Chem.* 2015, 87, 8373–8380. [CrossRef] [PubMed]

80. Allen, J.; Davey, H.M.; Broadhurst, D.; Heald, J.K.; Rowland, J.J.; Oliver, S.G.; Kell, D.B. High-throughput classification of yeast mutants for functional genomics using metabolic footprinting. *Nat. Biotechnol.* 2003, 21, 692–696. [CrossRef] [PubMed]

81. Klose, C.; Tarasov, K. Profiling of yeast lipids by shotgun lipidomics. *Methods Mol. Biol.* 2016, 1361, 309–324. [PubMed]

82. Wang, C.; Wang, M.; Han, X. Comprehensive and quantitative analysis of lysophospholipid molecular species present in obese mouse liver by shotgun lipidomics. *Anal. Chem.* 2015, 87, 4879–4887. [CrossRef] [PubMed]

83. Surma, M.A.; Herzog, R.; Vasilj, A.; Klose, C.; Christinat, N.; Morin-Rivron, D.; Simons, K.; Masoodi, M.; Sampaio, J.L. An automated shotgun lipidomics platform for high throughput, comprehensive, and quantitative analysis of blood plasma intact lipids. *Eur. J. Lipid Sci. Technol.* 2015, 117, 1540–1549. [CrossRef] [PubMed]

84. Papan, C.; Penkov, S.; Herzog, R.; Thiele, C.; Kurzchalia, T.; Shevchenko, A. Systematic screening for novel lipids by shotgun lipidomics. *Anal. Chem.* 2014, 86, 2703–2710. [CrossRef] [PubMed]

85. Lintonen, T.P.; Baker, P.R.; Suoniemi, M.; Ubhi, B.K.; Koistinen, K.M.; Duchoslav, E.; Campbell, J.I.; Ekroos, K. Differential mobility spectrometry-driven shotgun lipidomics. *Anal. Chem.* 2014, 86, 9662–9669. [CrossRef] [PubMed]

86. Bhattacharya, S.K. Recent advances in shotgun lipidomics and their implication for vision research and ophthalmology. *Curr. Eye Res.* 2013, 38, 417–427. [CrossRef] [PubMed]

87. Wang, J.N.; Zhou, Y.; Guo, Y.L. Prediction of acute cellular renal allograft rejection by urinary metabolomics using MALDI-FTMS. *J. Proteome Res.* 2008, 7, 3597–3601. [CrossRef] [PubMed]

88. Wang, J.; Zhou, Y.; Guo, Y.; Zhu, T. Urinary metabolomics in monitoring acute tubular injury of renal allografts: A preliminary report. *Transplant. Proc.* 2011, 43, 3738–3742. [CrossRef] [PubMed]

89. Grata, E.; Boccard, J.; Glauser, G.; Courtois, P.A.; Farmer, E.E.; Wolfender, J.L.; Rudaz, S. Development of a two-step screening ESI-TOF-MS method for rapid determination of significant stress-induced metabolome modifications in plant leaf extracts: The wound response in Arabidopsis thaliana as a case study. *J. Sep. Sci.* 2007, 30, 2268–2278. [CrossRef] [PubMed]

90. Shuman, J.L.; Cortes, D.F.; Armenta, J.M.; Mendes, P.; Shulaev, V. Plant metabolomics by GC-MS and differential analysis. *Methods Mol. Biol.* 2011, 678, 229–246. [PubMed]

91. Shulaev, V.; Cortes, D.; Miller, G.; Mittler, R. Metabolomics for plant stress response. *Physiol. Plant.* 2008, 132, 199–208. [CrossRef] [PubMed]

92. Shulaev, V. Metabolomics technology and bioinformatics. *Brief. Bioinform.* 2006, 7, 128–139. [CrossRef] [PubMed]
93. Hu, C.; van Dommelen, J.; van der Heijden, R.; Spijksma, G.; Reijmers, T.H.; Wang, M.; Slee, E.; Lu, X.; Xu, G.; van der Gref, J.; et al. RPLC-ion-trap-FTMS method for lipid profiling of plasma: Method validation and application to p53 mutant mouse model. *J. Proteome Res.* 2008, 7, 4982–4991. [CrossRef] [PubMed]

94. Hummel, J.; Segu, S.; Li, Y.; Irgan, S.; Jueppner, J.; Giavalisco, P. Ultra performance liquid chromatography and high resolution mass spectrometry for the analysis of plant lipids. *Front. Plant Sci.* 2011, 2. [CrossRef] [PubMed]

95. Alexandre-Gouabau, M.C.; Courant, F.; Moyer, T.; Kuster, A.; le Gall, G.; Tea, I.; Antignac, J.P.; Darmaun, D. Maternal and cord blood LC-HRMS metabolomics reveal alterations in energy and polyamine metabolism, and oxidative stress in very-low birth weight infants. *J. Proteome Res.* 2013, 12, 2764–2778. [CrossRef] [PubMed]

96. Bessonneau, V.; Bojko, B.; Pawliszyn, J. Analysis of human saliva metabolome by direct immersion solid-phase microextraction LC and benchtop orbitrap MS. *Bioanalysis* 2013, 5, 783–792. [CrossRef] [PubMed]

97. Bhatnagar, D.; de Saeger, S. Use of UHPLC high-resolution Orbitrap mass spectrometry to investigate the genes involved in the production of secondary metabolites in *Aspergillus flavus*. *Food Addit. Contam. Part A* 2015, 32, 1656–1673. [CrossRef] [PubMed]

98. Boyard-Kieken, F.; Dervilly-Pinel, G.; Garcia, P.; Paris, A.C.; Popot, M.A.; le Bizec, B.; Bonnaire, Y. Comparison of different liquid chromatography stationary phases in LC-HRMS metabolomics for the detection of recombinant growth hormone doping control. *J. Sep. Sci.* 2011, 34, 3493–3501. [CrossRef] [PubMed]

99. Bueschl, C.; Kraska, R.; Kluger, B. Isotopic labeling-assisted metabolomics using LC-MS. *Anal. Bioanal. Chem.* 2013, 405, 27–33. [CrossRef] [PubMed]

100. Du, L.N.; Xie, T.; Xu, J.Y.; Kang, A.; Di, L.Q.; Shan, J.; Wang, S.C. A metabolomics approach to studying the effects of Jinxin oral liquid on RSV-infected mice using UPLC/LTQ-Orbitrap mass spectrometry. *J. Chromatogr. B* 2008, 871, 306–313. [CrossRef] [PubMed]

101. Guo, X.; Lankmayr, E. Multidimensional approaches in LC and MS for phospholipid bioanalysis. *Bioanalysis* 2010, 2, 1109–1123. [CrossRef] [PubMed]

102. Gertsman, I.; Gangoiti, J.A.; Barshop, B.A. Validation of a dual LC-HRMS platform for clinical metabolic diagnosis in serum, bridging quantitative analysis and untargeted metabolomics. *Metabolomics* 2014, 10, 312–322. [CrossRef] [PubMed]

103. Hu, C.; van Dommelen, J.; van der Heijden, R.; Spijksma, G.; Reijmers, T.H.; Wang, M.; Slee, E.; Lu, X.; Xu, G.; van der Gref, J.; et al. RPLC-ion-trap-FTMS method for lipid profiling of plasma: Method validation and application to p53 mutant mouse model. *J. Proteome Res.* 2008, 7, 4982–4991. [CrossRef] [PubMed]

104. Kieken, F.; Pinel, G.; Garcia, P.; Paris, A.C.; Popot, M.A.; le Bizec, B.; Bonnaire, Y. Comparison of different liquid chromatography stationary phases in LC-HRMS metabolomics for the detection of recombinant growth hormone doping control. *J. Sep. Sci.* 2011, 34, 3493–3501. [CrossRef] [PubMed]

105. Kluger, B.; Kerska, R.; Krska, R.; Kluger, B.; Neumann, N.; Stuckler, R.; Doppler, M.; Chassy, A.W.; Waterhouse, A.L.; Rechthaler, J.; et al. Untargeted profiling of tracer-derived metabolites using stable isotopic labeling and fast polarity-switching LC-ESI-HRMS. *Anal. Chem.* 2014, 86, 11533–11537. [CrossRef] [PubMed]

106. Kokkotou, K.; Ioannou, E.; Nomikou, M.; Pitterl, F.; Vonaparti, A.; Siapi, E.; Zervou, M.; Roussis, V. An integrated approach using UHPLC-PDA-HRMS and 2D HSQC NMR for the metabolic profiling of the red alga Laurencia: Dereplication and tracing of natural products. *Phytochemistry* 2014, 108, 208–219. [CrossRef] [PubMed]

107. Li, L.; Zhang, F.; Zaia, J.; Linhardt, R.J. Top-down approach for the direct characterization of low molecular weight heparins using LC-FT-MS. *Anal. Chem.* 2012, 84, 8822–8829. [CrossRef] [PubMed]

108. Lu, W.; Bennett, B.D.; Rabinowitz, J.D. Analytical strategies for LC-MS-based targeted metabolomics. *J. Chromatogr. B* 2008, 871, 236–242. [CrossRef] [PubMed]

109. Madji Hounoum, B.; Blasco, H.; Nadal-Desbarats, L.; Dieme, B.; Montigny, F.; Andres, C.R.; Emond, P.; Mavel, S. Analytical methodology for metabolomics study of adherent mammalian cells using NMR, GC-MS and LC-HRMS. *Anal. Bioanal. Chem.* 2015, 407, 8861–8872. [CrossRef] [PubMed]
110. Nemkov, T.; D’Alessandro, A.; Hansen, K.C. Three-minute method for amino acid analysis by UHPLC and high-resolution quadrupole orbitrap mass spectrometry. *Amino Acids* 2015, 47, 2345–2357. [CrossRef] [PubMed]

111. Neumann, N.K.; Lehner, S.M.; Kluger, B.; Bueschl, C.; Sedelmaier, K.; Lemmens, M.; Kraska, R.; Schuhmacher, R. Automated LC-HRMS/(MS) approach for the annotation of fragment ions derived from stable isotope labeling-assisted untargeted metabolomics. *Anal. Chem.* 2014, 86, 7320–7327. [CrossRef] [PubMed]

112. Ni, Z.; Milic, I.; Fedorova, M. Identification of carbonylated lipids from different phospholipid classes by shotgun and LC-MS lipidomics. *Anal. Bioanal. Chem.* 2015, 407, 5161–5173. [CrossRef] [PubMed]

113. Orellana, G.; Vanden Bussche, J.; van Meulebroek, L.; Vandegehuchte, M.; Janssen, C.; Vanhaecke, L. Validation of a confirmatory method for lipophilic marine toxins in shellfish using UHPLC-HR-Orbitrap MS. *Anal. Bioanal. Chem.* 2014, 406, 5303–5312. [CrossRef] [PubMed]

114. Rochat, B. Quantitative/qualitative analysis using LC-HRMS: The fundamental step forward for clinical laboratories and clinical practice. *Bioanalysis* 2012, 4, 1709–1711. [CrossRef] [PubMed]

115. Stojiljkovic, N.; Paris, A.; Garcia, P.; Popot, M.A.; Bonnaire, Y.; Tabet, J.C.; Junot, C. Evaluation of horse urine sample preparation methods for metabolomics using LC coupled to HRMS. *Bioanalysis* 2014, 6, 785–803. [CrossRef] [PubMed]

116. Takahashi, H.; Morimoto, T.; Ogasawara, N.; Kanaya, S. AMDORAP: Non-targeted metabolic profiling based on high-resolution LC-MS. *BMC Bioinform.* 2011, 12, 259. [CrossRef] [PubMed]

117. Zeng, Z.; Liu, X.; Dai, W.; Yin, P.; Zhou, L.; Huang, Q.; Lin, X.; Xu, G. Ion fusion of high-resolution LC-MS-based metabolomics data to discover more reliable biomarkers. *Anal. Chem.* 2014, 86, 3793–3800. [CrossRef] [PubMed]

118. Zhou, T.; Wang, M.; Cheng, H.; Cui, C.; Su, S.; Xu, P.; Xue, M. UPLC-HRMS based metabolomics reveals the sphingolipids with long fatty chains and olefinic bonds up-regulated in metabolic pathway for hypoxia preconditioning. *Chem. Biol. Interact.* 2015, 242, 145–152. [CrossRef] [PubMed]

119. Buck, A.; Ly, A.; Balluff, B.; Sun, N.; Gorzolka, K.; Weirich, G.; et al. High-resolution MALDI-FT-ICR MS imaging for the analysis of metabolites from formalin-fixed, paraffin-embedded clinical tissue samples. *J. Pathol.* 2015, 237, 123–132. [CrossRef] [PubMed]

120. Jirasko, R.; Holcapek, M.; Kunes, M.; Svatos, A. Distribution study of atorvastatin and its metabolites in rat tissues using combined information from UHPLC/MS and MALDI-Orbitrap-MS imaging. *Anal. Bioanal. Chem.* 2014, 406, 4601–4610. [CrossRef] [PubMed]
Int. J. Mol. Sci. 2016, 17, 816

128. Kim, Y.H.; Fujimura, Y.; Sasaki, M.; Yang, X.; Yukihiro, D.; Miura, D.; Unno, Y.; Ogata, K.; Nakajima, H.; Yamashita, S.; et al. In situ label-free visualization of orally dosed strictinin within mouse kidney by MALDI-MS imaging. *J. Agric. Food Chem.* 2014, 62, 9279–9285. [CrossRef] [PubMed]

129. Park, E.S.; Lee, J.H.; Hong, J.H.; Park, Y.K.; Lee, J.W.; Lee, W.J.; Kim, K.P.; Kim, K.H. Phosphatidylcholine alteration identified using MALDI imaging MS in HBV-infected mouse livers and virus-mediated regeneration defects. *PLoS ONE* 2014, 9, e103955. [CrossRef] [PubMed]

130. Schulz, S.; Gerhardt, D.; Meyer, B.; Seegel, M.; Schubach, B.; Hofp, C.; Matheis, K. DMSO-enhanced MALDI MS imaging with normalization against a deuterated standard for relative quantification of dasatinib in serial mouse pharmacology studies. *Anal. Bioanal. Chem.* 2013, 405, 9467–9476. [CrossRef] [PubMed]

131. Berry, K.A.; Li, B.; Reynolds, S.D.; Barkley, R.M.; Gijon, M.A.; Hankin, J.A.; Murphy, R.C. MALDI imaging MS of phospholipids in the mouse lung. *J. Lipid Res.* 2011, 52, 1551–1560. [CrossRef]

132. Kusari, S.; Sezgin, S.; Nigutova, K.; Cellarova, E.; Spiteller, M. Spatial chemo-profiling of hypericin and related phytochemicals in *Hypericum* species using MALDI-HRMS imaging. *Anal. Bioanal. Chem.* 2015, 407, 4779–4791. [CrossRef] [PubMed]

133. Horn, P.J.; Chapman, K.D. Lipidomics in situ: Insights into plant lipid metabolism from high resolution spatial maps of metabolites. *Prog. Lipid Res.* 2014, 54, 32–52. [CrossRef] [PubMed]

134. Horn, P.J.; Chapman, K.D. Matrix assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) for direct visualization of plant metabolites in situ. *Curr Opin Biotechnol.* 2014, 54, 32–52. [CrossRef] [PubMed]

135. Sturtevant, D.; Lee, Y.J.; Chapman, K.D. Matrix assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) for direct visualization of plant metabolites in situ. *Curr Opin Biotechnol.* 2016, 37, 53–60. [CrossRef] [PubMed]

136. Touboul, D.; Brunelle, A. MALDI mass spectrometry imaging of lipids and primary metabolites on rat brain sections. *Methods Mol. Biol.* 2015, 1203, 41–48. [PubMed]

137. Jadoul, L.; Longuespee, R.; Noel, A.; de Pauw, E. A spiked tissue-based approach for quantification of phosphatidylcholines in brain section by MALDI mass spectrometry imaging. *Anal. Bioanal. Chem.* 2015, 407, 2095–2106. [CrossRef] [PubMed]

138. Holcapek, M.; Cervena, B.; Cifkova, E.; Lisa, M.; Chagovets, V.; Vostalova, J.; Bancirova, M.; Galuszka, J.; Hill, M. Lipidomic analysis of plasma, erythrocytes and lipoprotein fractions of cardiovascular disease patients using UHPLC/MS, MALDI-MS and multivariate data analysis. *J. Chromatogr. B* 2015, 990, 52–63. [CrossRef] [PubMed]

139. Wei, Y.; Zhang, Y.; Lin, Y.; Li, L.; Liu, J.; Wang, Z.; Xiong, S.; Zhao, Z. A uniform 2,5-dihydroxybenzoic acid layer as a matrix for MALDI-FTICR MS-based lipidomics. *Analyst* 2015, 140, 1298–1305. [CrossRef] [PubMed]

140. Ly, A.; Schone, C.; Becker, M.; Rattke, J.; Meding, S.; Aichler, M.; Suckau, D.; Walch, A.; Hauck, S.M.; Ueffing, M. High-resolution MALDI mass spectrometric imaging of lipids in the mammalian retina. *Histochem. Cell Biol.* 2015, 143, 453–462. [CrossRef]

141. Wildburger, N.C.; Wood, P.L.; Gumin, J.; Lichti, C.F.; Emmett, M.R.; Lang, F.F.; Nilsson, C.L. ESI-MS/MS and MALDI-IMS localization reveal alterations in phosphatidic acid, diacylglycerol, and DHA in glioma stem cell xenografts. *J. Proteome Res.* 2015, 14, 2511–2519. [CrossRef] [PubMed]

142. Holcapek, M.; Cervena, B.; Cifkova, E.; Lisa, M.; Chagovets, V.; Vostalova, J.; Bancirova, M.; Galuszka, J.; Hill, M. Lipidomic analysis of plasma, erythrocytes and lipoprotein fractions of cardiovascular disease patients using UHPLC/MS, MALDI-MS and multivariate data analysis. *J. Chromatogr. B* 2015, 990, 52–63. [CrossRef] [PubMed]

143. Xu, L.; Kliman, M.; Forsythe, J.G.; Korade, Z.; Hmelo, A.B.; Porter, N.A.; McLean, J.A. Profiling and imaging ion mobility-mass spectrometry analysis of cholesterol and 7-dehydrocholesterol in cells via sputtered silver MALDI. *J. Am. Soc. Mass Spectrom.* 2015, 26, 924–933. [CrossRef] [PubMed]

144. Korte, A.R.; Yandeau-Nelson, M.D.; Nikolau, B.J.; Lee, Y.J. Subcellular-level resolution MALDI-MS imaging of maize leaf metabolites by MALDI-linear ion trap-Orbitrap mass spectrometer. *Anal. Bioanal. Chem.* 2015, 407, 2301–2309. [CrossRef] [PubMed]
145. Gemperline, E.; Jayaraman, D.; Maeda, J.; Ane, J.M.; Li, L. Multifaceted investigation of metabolites during nitrogen fixation in Medicago via high resolution MALDI-MS imaging and ESI-MS. *J. Am. Soc. Mass Spectrom.* 2015, 26, 149–158. [CrossRef] [PubMed]

146. Barry, J.A.; Groseclose, M.R.; Robichaud, G.; Castellino, S.; Muddiman, D.C. Assessing drug and metabolite detection in liver tissue by UV-MALDI and IR-MALDESI mass spectrometry imaging coupled to FT-ICR MS. *Int. J. Mass Spectrom.* 2015, 377, 448–155. [CrossRef] [PubMed]

147. Bhandari, D.R.; Shen, T.; Rompp, A.; Zorn, H.; Spengler, B. Analysis of cyathane-type diterpenoids from *Cyathus striatus* and *Hericium Erinaceus* by high-resolution MALDI MS imaging. *Anal. Bioanal. Chem. 2014, 406, 695–704. [CrossRef] [PubMed]

148. Spengler, B.; Hubert, M. Scanning microprobe matrix-assisted laser desorption ionization (MALDI) mass spectrometry: Instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *J. Am. Soc. Mass Spectrom. 2002, 13, 735–748. [CrossRef]

149. Bhandari, D.R.; Schott, M.; Rompp, A.; Vilcinskas, A.; Spengler, B. Metabolite localization by atmospheric pressure high-resolution scanning microprobe matrix-assisted laser desorption/ ionization mass spectrometry imaging in whole-body sections and individual organs of the rove beetle *Paederus riparius*. *Anal. Bioanal. Chem. 2015, 407, 2189–2201. [CrossRef] [PubMed]

150. Schober, Y.; Schramm, T.; Spengler, B.; Rompp, A. Protein identification by accurate mass matrix-assisted laser desorption/ionization imaging of tryptic peptides. *Rapid Commun. Mass Spectrom.* 2011, 25, 2475–2483. [CrossRef] [PubMed]

151. Rompp, A.; Guenther, S.; Takats, Z.; Spengler, B. Mass spectrometry imaging with high resolution in mass and space (HR² MSI) for reliable investigation of drug compound distributions on the cellular level. *Anal. Bioanal. Chem. 2011, 401, 65–73. [CrossRef] [PubMed]

152. Comi, T.J.; Ryu, S.W.; Perry, R.H. Synchronized desorption electrospray ionization mass spectrometry imaging. *Anal. Chem. 2016, 88, 1169–1175. [CrossRef] [PubMed]

153. Barry, J.A.; Groseclose, M.R.; Robichaud, G.; Garrard, K.P.; Muddiman, D.C. Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) imaging source coupled to a FT-ICR mass spectrometer. *J. Am. Soc. Mass Spectrom. 2013, 24, 92–100. [CrossRef] [PubMed]

154. Barry, J.A.; Muddiman, D.C. Global optimization of the infrared matrix-assisted laser desorption electrospray ionization (IR MALDESI) source for mass spectrometry using statistical design of experiments. *Rapid Commun. Mass Spectrom. 2011, 25, 3527–3536. [CrossRef] [PubMed]

155. Ojanperä, I.; Kolmonen, M.; Pelander, A. Current use of high-resolution mass spectrometry in drug screening relevant to clinical and forensic toxicology and doping control. *Anal. Bioanal. Chem. 2012, 403, 1203–1220. [CrossRef] [PubMed]

156. Wu, A.H.; Gerona, R.; Armenian, P.; French, D.; Petrie, M.; Lynch, K.L. Role of liquid chromatography-high-resolution mass spectrometry (LC-HR/MS) in clinical toxicology. *Clin. Toxicol. 2012, 50, 733–742. [CrossRef] [PubMed]

157. Martínez-Domínguez, G.; Romero-Gonzalez, R.; Garrido Frenich, A. Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Food Chem. 2016, 197, 907–915. [CrossRef] [PubMed]

158. Dzuman, Z.; Zachariasova, M.; Veprikova, Z.; Godula, M.; Hajslova, J. Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids. *Anal. Chim. Acta 2015, 863, 29–40. [CrossRef] [PubMed]

159. De Dominicis, E.; Comissatti, I.; Suman, M. Targeted screening of pesticides, veterinary drugs and mycotoxins in bakery ingredients and food commodities by liquid chromatography-high-resolution single-stage Orbitrap mass spectrometry. *J. Mass Spectrom. 2012, 47, 1232–1241. [CrossRef] [PubMed]

160. Lattanzio, V.M.; Gatta, S.D.; Godula, M.; Visconti, A. Quantitative analysis of mycotoxins in cereal foods by collision cell fragmentation-high-resolution mass spectrometry: Performance and comparison with triple-stage quadrupole detection. *Food Addit. Contam. Part A 2011, 28, 1424–1437. [CrossRef] [PubMed]
163. Turnipseed, S.B.; Lohne, J.J.; Boisen, J.O. Review: Application of high resolution mass spectrometry to monitor veterinary drug residues in aquacultured products. J. AOAC Int. 2015, 98, 550–588. [PubMed]

164. Munoz, K.; Schmidt-Heydt, M.; Stoll, D.; Diehl, D.; Ziegler, J.; Geisen, R.; Schaumann, G.E. Effect of plastic mulching on mycotoxin occurrence and mycobiome abundance in soil samples from asparagus crops. Mycotoxin Res. 2015, 31, 191–201. [CrossRef] [PubMed]

165. Lattanzio, V.M.; Ciasca, B.; Terzi, V.; Ghizzoni, R.; McCormick, S.P.; Pascale, M. Study of the natural occurrence of T-2 and HT-2 toxins and their glucosyl derivatives from field barley to malt by high-resolution Orbitrap mass spectrometry. Food Addit. Contam. Part A 2015, 32, 1647–1655. [CrossRef] [PubMed]

166. Kelman, M.J.; Renaud, J.B.; Seifert, K.A.; Mack, J.; Sivagnanam, K.; Yeung, K.K.; Sumarah, M.W. Identification of six new Alternaria sulfoconjugated metabolites by high-resolution neutral loss filtering. Rapid Commun. Mass Spectrom. 2015, 29, 1805–1810. [CrossRef] [PubMed]

167. Deng, Y.Y.; Jia, L.J.; Zhang, K.; Yin, H.W. Combinatorial biochemical and chemical analyses of polychlorinated dibenzo-p-dioxins and dibenzofurans in agricultural soils from Chongming Island, Shanghai, China. Bull. Environ. Contam. Toxicol. 2015, 94, 183–187. [CrossRef] [PubMed]

168. Kiss, A.; Lucio, M.; Fildier, A.; Buisson, C.; Schmitt-Kopplin, P.; Cren-Olive, C. Doping control using high and ultra-high resolution mass spectrometry based non-targeted metabolomics—a case study of salbutamol and budesonide abuse. PLoS ONE 2013, 8, e74584. [CrossRef] [PubMed]

169. Sun, J.; Liu, X.; Yang, T.; Slovin, J.; Chen, P. Profiling polyphenols of two diploid strawberry (Fragaria vesca) inbred lines using UHPLC-HRMS. Food Chem. 2014, 146, 289–298. [CrossRef] [PubMed]

170. Walker, A.; Lucio, M.; Pfitzner, B.; Scheerer, M.F.; Neschen, S.; de Angelis, M.H.; Hartmann, A.; Schmitt-Kopplin, P. Importance of sulfur-containing metabolites in discriminating fecal extracts between normal and type-2 diabetic mice. J. Proteome Res. 2014, 13, 4220–4231. [CrossRef] [PubMed]

171. Walker, A.; Pfitzner, B.; Neschen, S.; Kahle, M.; Harir, M.; Lucio, M.; Moritz, F.; Tziotis, D.; Witting, M.; Rothballer, M.; et al. Distinct signatures of host-microbial meta-metabolome and gut microbiome in two C57BL/6 strains under high-fat diet. ISME J. 2014, 8, 2380–2396. [CrossRef] [PubMed]

172. Nacher-Mestre, J.; Ibanez, M.; Serrano, R.; Perez-Sanchez, J.; Hernandez, F. Qualitative screening of undesirable compounds from feeds to fish by liquid chromatography coupled to mass spectrometry. J. Agric. Food Chem. 2013, 61, 2077–2087. [CrossRef] [PubMed]

173. Winkler, J. High levels of dioxin-like PCBs found in organic-farmed eggs caused by coating materials of asbestos-cement fiber plates: A case study. Environ. Int. 2015, 80, 72–78. [CrossRef] [PubMed]

174. Solliec, M.; Roy-Lachapelle, A.; Sauve, S. Quantitative performance of liquid chromatography coupled to Q-Exactive high resolution mass spectrometry (HRMS) for the analysis of tetracyclines in a complex matrix. Anal. Chim. Acta 2015, 853, 415–424. [CrossRef] [PubMed]

175. Seiwert, B.; Golan-Rozen, N.; Weidauer, C.; Riemenschneider, C.; Chefetz, B.; Hadar, Y.; Reemtsma, T. Electrochemistry combined with LC-HRMS: Elucidating transformation products of the recalcitrant pharmaceutical compound carbamazepine generated by the white-rot fungus Pleurotus ostreatus. Environ. Sci. Technol. 2015, 49, 12342–12350. [CrossRef] [PubMed]

176. Matsumoto, R.; Tu, N.P.; Haruta, S.; Kawano, M.; Takeuchi, I. Polychlorinated biphenyl (PCB) concentrations and congener composition in masu salmon from Japan: A study of all 209 PCB congeners by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Mar. Pollut. Bull. 2014, 85, 549–557. [CrossRef] [PubMed]

177. Kakimoto, K.; Nagayoshi, H.; Konishi, Y.; Kajimura, K.; Ohura, T.; Hayakawa, K.; Toriba, A. Atmospheric chlorinated polycyclic aromatic hydrocarbons in East Asia. Chemosphere 2014, 111, 40–46. [CrossRef] [PubMed]

178. Song, Y.; Wu, N.; Han, J.; Shen, H.; Tan, Y.; Ding, G.; Xiang, J.; Tao, H.; Jin, S. Levels of PCDD/Fs and DL-PCBs in selected foods and estimated dietary intake for the local residents of Luqiao and Yuhang in Zhejiang, China. Chemosphere 2011, 85, 329–334. [CrossRef] [PubMed]

179. Woudneh, M.B.; Ou, Z.; Sekela, M.; Tuominen, T.; Gledhill, M. Pesticide multiresidues in waters of the Lower Fraser Valley, British Columbia, Canada. Part I. Surface water. J. Environ. Qual. 2009, 38, 940–947. [CrossRef] [PubMed]

180. Katajamaa, M.; Oresic, M. Data processing for mass spectrometry-based metabolomics. J Chromatogr A 2007, 1158, 318–326. [CrossRef] [PubMed]
181. Madsen, R.; Lundstedt, T.; Trygg, J. Chemometrics in metabolomics—A review in human disease diagnosis. *Anal. Chim. Acta* 2010, 659, 23–33. [CrossRef] [PubMed]

182. Blekherman, G.; Laubenbacher, R.; Cortes, D.F.; Mendes, P.; Torti, F.M.; Akman, S.; Torti, S.V.; Shulaev, V. Bioinformatics tools for cancer metabolomics. *Metabolomics* 2011, 7, 329–343. [CrossRef] [PubMed]

183. Markley, J.L.; Anderson, M.E.; Cui, Q.; Egblinialia, H.R.; Lewis, I.A.; Hegeman, A.D.; Li, J.; Schulte, C.F.; Sussman, M.R.; Westler, W.M.; *et al.* New bioinformatics resources for metabolomics. *Pac. Symp. Biocomput.* 2007, 12, 157–168.

184. Gougeon, R.D.; Lucio, M.; Frommberger, M.; Peyron, D.; Chassagne, D.; Alexandre, H.; Feuillat, F.; Voilley, A.; Cayot, P.; Gebefugi, I.; *et al.* The chemodiversity of wines can reveal a metabologeography expression of cooperage oak wood. *Proc. Natl. Acad. Sci. USA* 2009, 106, 9174–9179. [CrossRef] [PubMed]

185. Roullier-Gall, C.; Witting, M.; Tziotis, D.; Ruf, A.; Gougeon, R.D.; Schmitt-Kopplin, P. Integrating analytical resolutions in non-targeted wine metabolomics. *Tetrahedron* 2015, 71, 2983–2990. [CrossRef]

186. Longnecker, K.; Futrelle, J.; Coburn, E.; Kido Soule, M.C.; Kujawinski, E.B. Environmental metabolomics: Databases and tools for data analysis. *Mar. Chem.* 2015, 177, 366–373. [CrossRef]

187. Roux, A.; Xu, Y.; Heilier, J.F.; Olivier, M.F.; Ezan, E.; Tabet, J.C.; Junot, C. Annotation of the human adult urinary metabolome and metabolite identification using ultra high performance liquid chromatography coupled to a linear quadrupole ion trap-Orbitrap mass spectrometer. *Anal. Chem.* 2012, 84, 6429–6437. [CrossRef] [PubMed]

188. Far, J.; Delvaux, C.; Kune, C.; Eppe, G.; de Pauw, E. The use of ion mobility mass spectrometry for isomer composition determination extracted from Se-rich yeast. *Anal. Chem.* 2014, 86, 11246–11254. [CrossRef] [PubMed]

189. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; *et al.* Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* 2007, 3, 211–221. [CrossRef] [PubMed]

190. Bolton, E.E.; Wang, Y.; Thiessen, P.A.; Bryant, S.H. Chapter 12—PubChem: Integrated platform of small molecules and biological activities. In *Annual Reports in Computational Chemistry*; Ralph, A.W., David, C.S., Eds.; Elsevier: Amsterdam, The Netherlands, 2008; Volume 4, pp. 217–241.

191. Kim, S.; Thiessen, P.A.; Bolton, E.E.; Chen, J.; Fu, G.; Gindulyte, A.; Han, L.; He, J.; He, S.; Shoemaker, B.A.; *et al.* PubChem substance and compound databases. *Nucleic Acids Res.* 2016, 44, D1202–D1213. [CrossRef] [PubMed]

192. The PubChem Project. Available online: https://pubchem.ncbi.nlm.nih.gov (accessed on 24 May 2016).

193. Little, J.L.; Williams, A.J.; Pshenichnov, A.; Tkachenko, V. Identification of “known unknowns” utilizing accurate mass data and ChemSpider. *J. Am. Soc. Mass Spectrom.* 2012, 23, 179–185. [CrossRef] [PubMed]

194. Pence, H.E.; Williams, A. ChemSpider: An online chemical information resource. *J. Chem. Educ.* 2010, 87, 1123–1124. [CrossRef]

195. ChemSpider. Available online: http://www.chemspider.com (accessed on 24 May 2016).

196. Scripps Center for Metabolomics and Mass Spectrometry. Available online: https://metlin.scripps.edu (accessed on 24 May 2016).

197. Wagele, B.; Witting, M.; Schmitt-Kopplin, P.; Suhre, K. MassTRIX reloaded: Combined analysis and visualization of transcriptome and metabolome data. *PLoS ONE* 2012, 7, e39860. [CrossRef] [PubMed]

198. Suhre, K.; Schmitt-Kopplin, P. MassTRIX: Mass translator into pathways. *Nucleic Acids Res.* 2008, 36, W481–W484. [CrossRef] [PubMed]
201. Narvaez-Rivas, M.; Zhang, Q. Comprehensive untargeted lipidomic analysis using core-shell C30 particle
202. Witting, M.; Schmitt-Kopplin, P. Transcriptome and metabolome data integration—Technical perquisites
203. Tziotis, D.; Hertkorn, N.; Schmitt-Kopplin, P. Kendrick-analogue network visualisation of ion cyclotron
204. Herzog, R.; Schwudke, D.; Eghbalnia, H.R.; Sussman, M.R.; Markley, J.L. Metabolite identification via the madison metabolomics consortium database. Nat. Biotechnol. 2008, 26, 162–164. [CrossRef] [PubMed]
205. Cui, Q.; Lewis, I.A.; Hegeman, A.D.; Anderson, M.E.; Li, J.; Schulte, C.F.; Westler, W.M.;
206. Forcisi, S.; Moritz, F.; Lucio, M.; Lehmann, R.; Stefan, N.; Schmitt-Kopplin, P. Solutions for low and high
207. Cui, Q.; Lewis, I.A.; Hegeman, A.D.; Anderson, M.E.; Li, J.; Schulte, C.F.; Anderson, M.E.; Li, J.; Schulte, C.F.; Westler, W.M.; Eghbalnia, H.R.; Sussman, M.R.; Markley, J.L. Metabolite identification via the madison metabolomics consortium database. Nat. Biotechnol. 2008, 26, 162–164. [CrossRef] [PubMed]
208. Wishart, D.S.; Knox, C.; Guo, A.C.; Eisner, R.; Young, N.; Gautam, B.; Hau, D.D.; Psychogios, N.; Dong, E.; Bouatra, S.; et al. HMDB: A knowledgebase for the human metabolome. Nucleic Acids Res. 2009, 37, D603–D610. [CrossRef] [PubMed]
209. Zhou, B.; Wang, J.; Ressom, H.W. MetaboSearch: Tool for mass-based metabolite identification using multiple databases. PLoS ONE 2012, 7, e40096. [CrossRef] [PubMed]
210. Fahy, E.; Sud, M.; Cotter, D.; Subramaniam, S. LIPID MAPS online tools for lipid research. Nucleic Acids Res. 2007, 35, W606–W612. [CrossRef] [PubMed]
211. Tziotis, D.; Hertkorn, N.; Schmitt-Kopplin, P. Kendrick-analogous network visualisation of ion cyclotron resonance Fourier transform mass spectra: Improved options for the assignment of elemental compositions and the classification of organic molecular complexity. Eur. J. Mass Spectrom. 2011, 17, 415–421. [CrossRef] [PubMed]
212. Tsiotis, D.; Hertkorn, N.; Schmitt-Kopplin, P. Kendrick-analogue network visualisation of ion cyclotron resonance Fourier transform mass spectra: Improved options for the assignment of elemental compositions and the classification of organic molecular complexity. Eur. J. Mass Spectrom. 2011, 17, 415–421. [CrossRef] [PubMed]
213. Taguchi, R.; Ishikawa, M. Precise and global identification of phospholipid molecular species by an Orbitrap mass spectrometer and automated search engine Lipid Search. J. Chromatogr. A 2010, 1217, 4229–4239. [CrossRef] [PubMed]
214. Herzog, R.; Schwudke, D.; Shevchenko, A. LipidXplorer: Software for quantitative shotgun lipidomics compatible with multiple mass spectrometry platforms. Curr. Protoc. Bioinform. 2013, 11, 14.12:1–14.12:30.
215. Narvaez-Rivas, M.; Zhang, Q. Comprehensive untargeted lipidomic analysis using core-shell C30 particle column and high field orbitrap mass spectrometer. J. Chromatogr. A 2016, 1440, 123–134. [CrossRef] [PubMed]
216. Breitkopf, S.B.; Yuan, M.; Helenius, K.P.; Lysiotis, C.A.; Asara, J.M. Triomics analysis of imatinib-treated myeloma cells connects kinase inhibition to RNA processing and decreased lipid biosynthesis. Anal. Chem. 2015, 87, D606–D612. [CrossRef] [PubMed]
217. Tafesse, F.G.; Rashidfarrokh, A.; Schmidt, F.I.; Freinkman, E.; Dougan, S.; Dougan, M.; Esteban, A.; Maruyama, T.; Strijbis, K.; Ploegh, H.L. Disruption of sphingolipid biosynthesis blocks phagocytosis of Candida albicans. PLoS Pathog. 2015, 11, e1005188. [CrossRef] [PubMed]
218. Trevino, M.B.; Machida, Y.; Hallinger, D.R.; Garcia, E.; Christensen, A.; Dutta, S.; Peake, D.A.; Ikeda, Y.; Imai, Y. Perilipin 5 regulates islet lipid metabolism and insulin secretion in a cAMP-dependent manner: Implication of its role in the postprandial insulin secretion. Diabetes 2015, 64, 1299–1310. [CrossRef] [PubMed]
219. Yamada, T.; Uchikata, T.; Sakamoto, S.; Yokoi, Y.; Fukusaki, E.; Bamba, T. Development of a lipid profiling system using reverse-phase liquid chromatography coupled to high-resolution mass spectrometry with rapid polarity switching and an automated lipid identification software. J. Chromatogr. A 2013, 1292, 211–218. [CrossRef] [PubMed]
220. Song, H.; Hsu, F.F.; Ladenson, J.; Turk, J. Algorithm for processing raw mass spectrometric data to identify and quantitate complex lipid molecular species in mixtures by data-dependent scanning and fragment ion database searching. J. Am. Soc. Mass Spectrom. 2007, 18, 1848–1858. [CrossRef] [PubMed]
221. Haimi, P.; Uphoff, A.; Hermansson, M.; Somerharju, P. Software tools for analysis of mass spectrometric lipidome data. *Anal. Chem.* 2006, 78, 8324–8331. [CrossRef] [PubMed]

222. Leavell, M.D.; Leary, J.A. Fatty acid analysis tool (FAAT): An FT-ICR MS lipid analysis algorithm. *Anal. Chem.* 2006, 78, 5497–5503. [CrossRef] [PubMed]

223. Hubner, G.; Crone, C.; Lindner, B. lipID—A software tool for automated assignment of lipids in mass spectra. *J. Mass Spectrom.* 2009, 44, 1676–1683. [CrossRef] [PubMed]

224. Song, H.; Ladenson, J.; Turk, J. Algorithms for automatic processing of data from mass spectrometric analyses of lipids. *J. Chromatogr. B* 2009, 877, 2847–2854. [CrossRef] [PubMed]

225. Kasper, P.T.; Rojas-Cherto, M.; Mistrik, R.; Reijmers, T.; Hankemeier, T.; Vreeken, R.J. Fragmentation trees for the structural characterisation of metabolites. *Rapid Commun. Mass Spectrom.* 2012, 26, 2275–2286. [CrossRef] [PubMed]

226. Sheldon, M.T.; Mistrik, R.; Croley, T.R. Determination of ion structures in structurally related compounds using precursor ion fingerprinting. *J. Am. Soc. Mass Spectrom.* 2009, 20, 370–376. [CrossRef] [PubMed]

227. mzCloud—Advanced Mass Spectral Database. Available online: https://www.mzcloud.org (accessed on 24 May 2016).

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