Journal of Chromatography B 1239 (2024) 124124

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Applications of chromatographic methods in metabolomics: A review

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ARTICLE INFO

Keywords:
Metabolomics
Liquid chromatography
Gas chromatography
Supercritical fluid chromatography
Mass spectrometry
Nuclear magnetic resonance spectroscopy

ABSTRACT

Chromatography is a robust and reliable separation method that can use various stationary phases to separate complex mixtures commonly seen in metabolomics. This review examines the types of chromatography and stationary phases that have been used in targeted or untargeted metabolomics with methods such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. General considerations for sample pretreatment and separations in metabolomics are considered, along with the various supports and separation formats for chromatography that have been used in such work. The types of liquid chromatography (LC) that have been most extensively used in metabolomics will be examined, such as reversed-phase liquid chromatography and hydrophilic liquid interaction chromatography. In addition, other forms of LC that have been used in more limited applications for metabolomics (*e.g.*, ion-exchange, size-exclusion, and affinity methods) will be discussed to illustrate how these techniques may be utilized for new and future research in this field. Multidimensional LC methods are also discussed, as well as the use of gas chromatography and supercritical fluid chromatography in metabolomics. In addition, the roles of chromatography in NMR- vs. MS-based metabolomics are considered. Applications are given within the field of metabolomics for each type of chromatography, along with potential advantages or limitations of these separation methods.

1. Introduction

Metabolomics is a field that involves the analysis of a metabolome, or a collection of low-mass compounds (typical mass, <1500 g/mol) that are produced through metabolic processes in a biological system [1–6]. Metabolites have diverse physical and chemical structures. Examples of structural classes of metabolites include small peptides, steroids, vitamins, carbohydrates, lipids, fatty acids, amino acids, and organic acids [1–9]. Many of these metabolites are the intermediates or end products of enzymatic reactions in biological systems and can be found in cells or tissues and complex biological fluids such as blood, urine, saliva, or cerebrospinal fluid [1–9].

Use of the term "metabolomics" began in the late 1990s [10–12]. Work in this field has grown significantly over the last few decades [2–8]. This growth is demonstrated in Fig. 1, which shows the number of publications that have appeared and included the terms "chromatography" plus "metabolomics" or "metabolites" between the years 2000 and 2022. As this figure indicates, chromatography has long been an important tool for the separation and analysis of metabolites (*e.g.,* in drug metabolism studies) and, as a result, has also been a key component in much of the work conducted in metabolomics [3,8]. In addition, the rapid growth in metabolomics over the last 20 years reflects the many areas that have been impacted by this field [13]. For instance, metabolomics has been used for drug discovery and development [14–16] and the detection or analysis of disease biomarkers [17,18]. Metabolomics has further been utilized as a tool in nutritional studies [19,20], animal breeding [21], plant biology [22], and characterization of the effects of various environmental factors on human health [15,23–27].

There are two major experimental approaches in metabolomics: targeted and untargeted methods [7,8,28,29]. Untargeted metabolomics, or global metabolomics, is based on a top-down strategy that ideally provides a comprehensive, unbiased analysis of all the metabolites in a biological sample [13,30]. This method is typically used in discovery-based research. The information that is obtained in untargeted metabolomics is used to compare patterns or fingerprints of metabolites, such as found in normal systems vs those that have been modified in response to an abnormal biological process, exposure to a drug, or a change due to genetic alterations in a biological system [31].

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https://doi.org/10.1016/j.jchromb.2024.124124
Received 3 October 2023; Received in revised form 11 March 2024; Accepted 10 April 2024
Available online 16 April 2024

Untargeted metabolomics has also been used to help identify metabolites that may act as biomarkers for disease states [16,32–35]. However, this approach can be challenging because of the time required to process the complex data that is generated, the need for adequate coverage of the metabolites in a system, and the requirement for accurate and reproducible measurements for many types of metabolites [27,36,37]. Targeted metabolomics is an alternative approach that is used to investigate a specific set of metabolites with related pathways of interest [7,28,38]. The emphasis on a given set of metabolites allows for better detection and absolute quantification of these compounds, as well as for more reproducible and stable conditions for data analysis. However, targeted metabolomics cannot detect unknown compounds and is generally used for only a limited number of metabolites [7,8,28,38].

One challenge in metabolomics is the diverse range of structures, properties, and concentrations that may occur for metabolites in biological samples [5,39,40]. At present, there is no single analytical method that can identify and detect the many types and levels of metabolites that may occur in living systems [41–51]. Instead, such work typically involves a combination of chromatography or a related separation method (*e.g.,* extractions) with a second analytical technique, which is usually nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) [4–6,52–61]. NMR has been used in just under a third of recent publications in metabolomics, with MS being employed in around 70 % of these reports [5,56]. In addition, some of these studies have used both NMR and MS [6,54,62,63].

Both NMR and MS have advantages and disadvantages when they are applied to metabolomics [6–9,28,29,57,61]. Advantages of utilizing NMR are the variety of information it can provide on compound structure, its good reproducibility, its relatively straightforward use for compound quantitation (*e.g.,* without the need for derivatization or calibration), its ability to work with metabolites that are not readily ionizable, and its need for only minimal sample manipulation [54]. These features have allowed NMR to often be used with only extractions or simple pretreatment steps besides chromatography; however, chromatography has also been used in a growing subset of this work, as is indicated in Fig. 2. In addition, hybrid methods such as liquid chromatography-NMR (LC-NMR) and LC-NMR-MS have been used for some research in metabolomics [54,57,62,63]. A key disadvantage of NMR is its sensitivity, which tends to limit this method in metabolomics to the study of relatively abundant metabolites (*i.e.,* those with concentrations >1 μM) [5]. The highly complex spectra and large amount of information provided by both NMR and MS (*e.g.*, in LC-MS) can also make it time consuming and challenging to process such data for the accurate identification and annotation of metabolites.

An advantage of MS is its higher sensitivity than NMR for detecting low-concentration metabolites [6–9,28,29,57]. The mass information that is provided by MS is also valuable in identifying compounds and in monitoring specific metabolites at low levels in biological matrices [28,29,41,42]. A possible limitation of MS when used in metabolomics is a general requirement for LC, gas chromatography (GC), or a related separation method [28,41,54,56,57,64–69]. This need is demonstrated in Fig. 2 by the large number of reports that have combined mass spectrometry with chromatography for metabolomics. The use of chromatography makes it possible for MS to distinguish and examine the many compounds that occur over a relatively narrow mass range in the metabolome [70] and to avoid effects by matrix components on ionization suppression [45].

Matrix effects can be an issue with both NMR and MS. The complex biological samples examined in metabolomics often require some level of pretreatment to minimize interferences from the sample matrix [5,28,40,41,43,45,52,53,57–59,71–73]. This often requires at least an extraction or separation step followed by filtration or centrifugation to remove cell/tissue debris and precipitated biomolecules. Again, more advanced separation or chromatographic techniques are typically

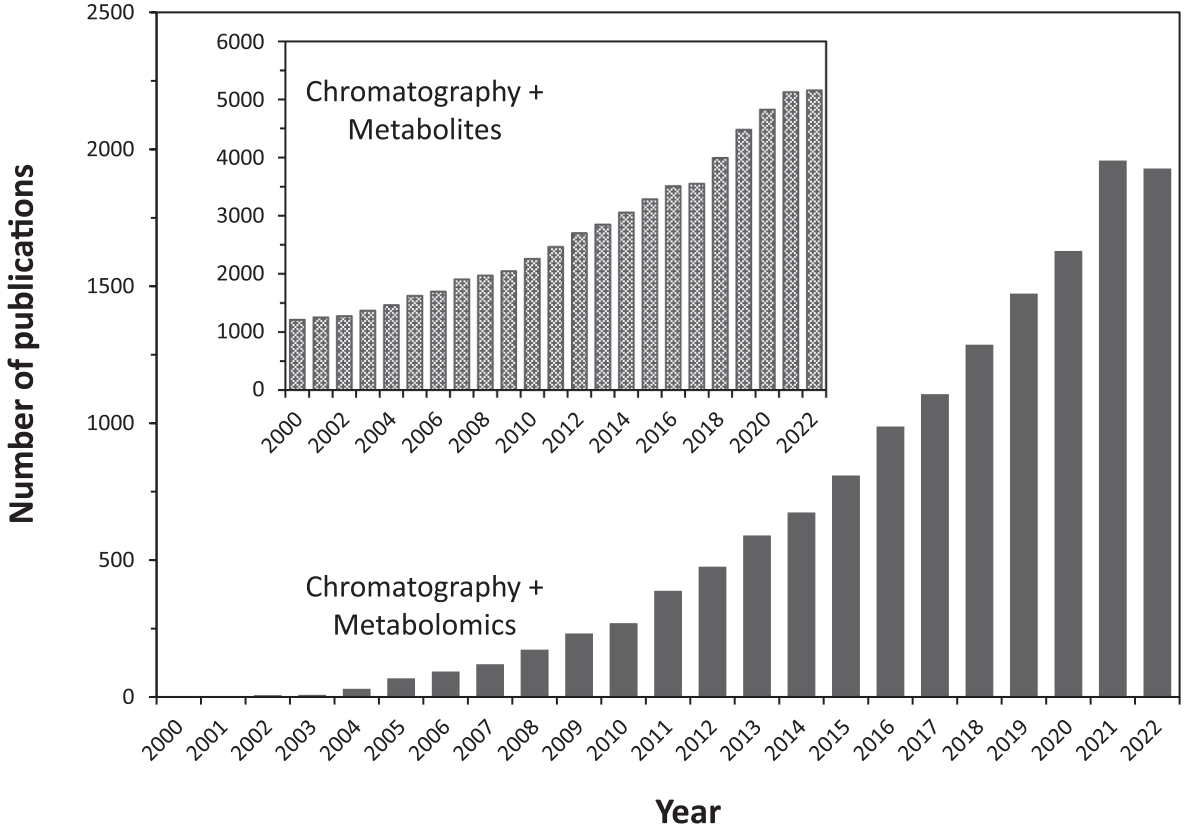

Fig. 1. Number of publications which included the terms “chromatography” and “metabolomics” or “metabolites” (inset) and which were published between 2000 and 2022. These results were obtained from searches conducted on the Web of Science in September 2023.

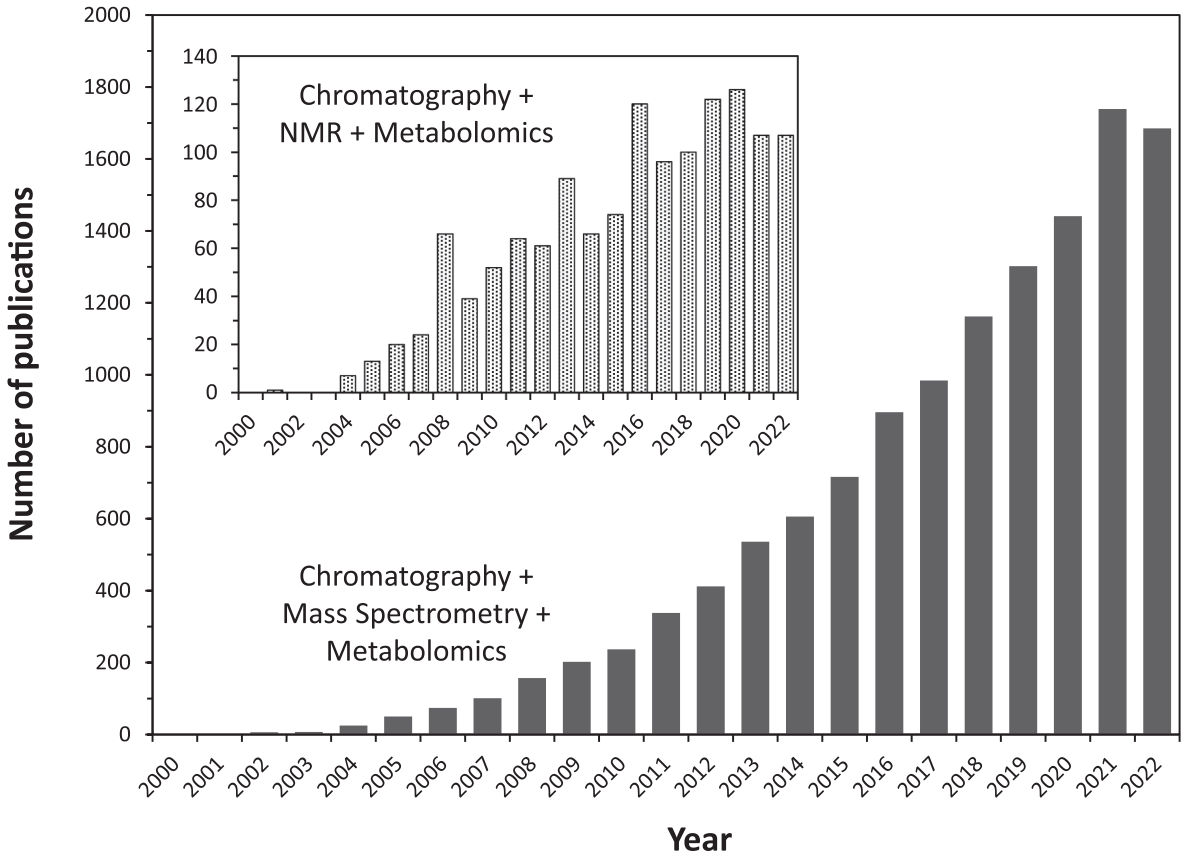

Fig. 2. Number of publications which included the terms "chromatography" and "metabolomics" plus "mass spectrometry" or "NMR" (inset) and which were published between 2000 and 2022. These results were obtained from searches conducted on the Web of Science in September 2023.

necessary for MS in metabolomics studies to enable reliable metabolite detection and annotation [45,64–69]. Although the application of direct-infusion MS methods to metabolomics has been reported [74,75], the use of direct-infusion vs. chromatography with MS for metabolomics is still a topic of debate in this field [76,77].

This review will examine the types of chromatography that have been used for sample separations or pretreatment in the field of metabolomics over the last two decades. A discussion will first be presented on how the different modes of LC have been employed and combined with techniques such as MS or NMR for metabolomics. This will include LC methods that are commonly used in metabolomics, such as reversed-phase liquid chromatography (RPLC) and hydrophilic liquid interaction chromatography (HILIC). In addition, other LC techniques will be discussed that have been used on a more limited basis but that may provide new opportunities for future research in this field. The use of methods such as GC, supercritical fluid chromatography (SFC), and planar chromatography will also be considered. Examples of applications will be provided for each of these techniques, along with a discussion of their potential advantages or limitations when employed in metabolomics.

2. General considerations in sample pretreatment for metabolomics

Sample pretreatment is often a key component in the workflow of an analytical method for metabolomics [16,46–49,60]. This step may involve sample clean-up, target enrichment, or analyte preconcentration to enhance the analysis of metabolites. A good sample pretreatment plan can minimize matrix effects, remove interfering compounds, and/or convert the metabolites of interest into a medium that is compatible with the methods of analysis that will be used to examine the sample's contents [45,47–49]. Although sample pretreatment and preliminary separations are often required in metabolomic studies, these steps can produce errors in such work (*e.g.*, through the creation of variations in MS ionization) [46–51]. In addition, some forms of sample pretreatment can be tedious and time-consuming to perform [16].

One factor to consider in sample pretreatment is whether the final analysis is to be used for untargeted vs. targeted metabolomics [35,46,78–80]. In untargeted metabolomics, the goal is to obtain a broad coverage of metabolites while reducing or eliminating loss of any compound; thus, only minimal sample pretreatment is desirable [31]. The sample preparation steps in this case should allow the recovery of a broad range of metabolites while also discarding biomolecules and sample debris [81,82]. Solvent extraction techniques often applied in metabolomics include the use of only water, 1:1 *v/v* water:methanol, or a Folch extraction consisting of a 3:4:8 *v/v* water:methanol:chloroform mixture [83,84]. It is also common to combine multiple separation steps and solvent conditions because two or three such steps may be necessary to maximize the recovery of all detectable metabolites [81–84]. In targeted metabolomics a more elaborate sample separation or pretreatment scheme may be needed, as the focus is instead on the analysis of only a given set of metabolites [35,46,78–80].

It is important that the pretreatment steps used in metabolomics provide a stable sample for analysis (*e.g.,* by quenching or removing enzymes that may alter the metabolites) and give a homogeneous sample for analysis that is an accurate representation of the relative levels and forms of the metabolites in their original matrix [60,85–88]. In addition, it is desirable to have a high rate of recovery of the desired metabolites with few interferences from other sample components. Proteins are examples of agents that may lead to significant interferences in metabolomics if they are not removed by sample

pretreatment [58,89–92]. For instance, proteins and other matrix components can alter the level of ionization and/or the overall apparent concentration of metabolites (*i.e.,* total vs. bound forms) during the use of LC with MS [35,46,54,56,78–80]. Proteins and sample components such as lipids can also affect spectral quality in NMR by producing broad background signals or additional peaks that may interfere in the detection and identification of metabolites [6,58,90–93].

3. Chromatographic supports and separation formats for metabolomics

Various supports and separation formats for chromatography have been employed for metabolomics. This is especially true for LC, the most common chromatographic method used in metabolomics. Early reports in metabolomics employed LC in a planar form by employing the method of thin-layer chromatography (TLC) [1,4,10–12,94,95]. The advantages of TLC are its ability to process several samples simultaneously, its low cost, and the relative ease with which it can be combined with other separation methods for multidimensional separations [96–99]. Early work in metabolomics utilized two-dimensional (2D) TLC to characterize metabolites in microorganisms and to study how variations in the concentrations of these metabolites affected cellular activity [1,4,10–12]. Several more recent studies have used TLC or high-performance TLC (or HPTLC) with imaging based on matrix-assisted laser desorption/ionization (MALDI) and MS for lipidomics (*i.e.,* a field closely related to metabolomics) [100–105]. Examples include the use of HPTLC with imaging based on MS to examine phospholipids in ovine bone marrow mesenchymal stem cells [102] and the use of TLC with imaging MS to profile extracted lipids from various portions of the human brain (see Fig. 3) [103]. HPTLC has also been employed with MS to monitor individual classes of lipids in human plasma [104] and lipids such as fatty acids, cholesterol, sphingolipids, and glycerophospholipids that were extracted from porcine brain [105].

The employment of LC as an on-line component in analytical methods for metabolomics is typically done by using packed columns in the methods of high-performance liquid chromatography (HPLC) or ultra-high-performance liquid chromatography (UHPLC, which is also referred to as ultra-performance liquid chromatography or UPLC) [66,69,106–128]. A few metabolomic studies using LC have also employed monolithic supports [129–131]. Modern HPLC columns are operated at pressures up to 5000–6000 psi and are typically packed with 3.5–5 μm diameter particles [41,97]. UHPLC is a subset of HPLC that uses smaller diameter particles (*i.e.,* less than 2 μm) and even higher operating pressures (*i.e.,* 17,400 psi) [97,124]. UHPLC has seen increasing use in metabolomics because it can provide a higher throughput with better resolution and sensitivity than conventional

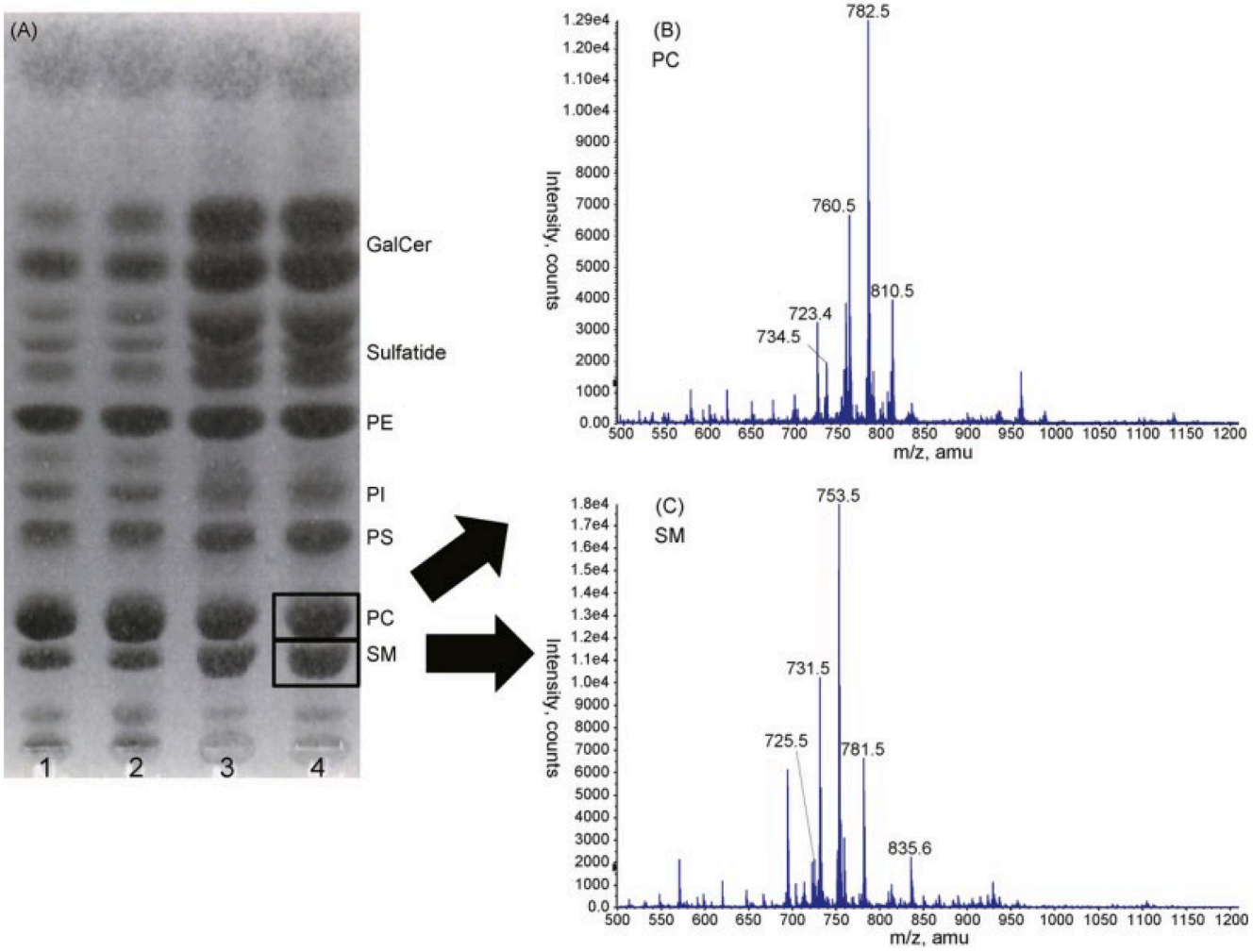

Fig. 3. (a) Separation of lipids from brain tissues by TLC, with visualization based on staining with primuline, and (b) mass spectra obtained for selected regions on the TLC plate, as illustrated here with spots obtained for phosphotidylcholine (PC) and sphingomyelin (SM). Other spots on this plate are for galactosylceramide (GalCer), sulfatide, phosphotidylethanolamine (PE), phosphotidylinositol (PI), and phosphotidylserine (PS). The four lanes in (a) represent results obtained with lipid extracts for the following regions of the human brain: (1) gray matter of the inferior frontal gyrus, (2) gray matter of the hippocampus, (3) white matter of the inferior frontal gyrus, and (4) white matter of hippocampus. This figure is reproduced from Ref. [103] with permission (Elsevier).

HPLC [125–127,132–136]. For instance, UHPLC gave a three-fold improvement in speed and an increase in signal-to-noise ratio for most of the examined compounds when both HPLC and UHPLC were used with MS, under comparable flow rate and mobile phase conditions, to analyze soy isoflavones or tamoxifen plus their corresponding metabolites [126]. This increase in sample throughput can be valuable for large-scale metabolomics studies, which can be comprised of hundreds to thousands of samples [137–139].

Current estimates suggest the metabolome is comprised of more than 270,000 compounds with a diverse set of chemical and physical properties [140]. At the simplest classification level, metabolites and lipids can be grouped into either polar or non-polar classes, which dictates the types of solvents and LC supports that are needed for the desired targets. This chemical complexity often leads to the use of several types of chromatographic separations [141–146], which is frequently referred to as a multidimensional separation method. Both 2D LC and GC have been utilized by the metabolomics community [99,147–150]. A single chromatographic method is insufficient for obtaining complete coverage of the metabolome. Instead, multidimensional separations (*e.g.,* based on LC) are commonly employed to increase the coverage of the metabolome and to account for the range of metabolites that can be present and which can vary widely in terms of their polarities and chemical classes [141,142].

A multidimensional chromatographic method can be created by combining two or more separate chromatographic steps either off-line or on-line with one another. A common example in the use of LC for metabolomics is the combination of RPLC with HILIC [120–122,151–158]. An off-line approach can be relatively simple to develop; however, this approach will probably also involve many manual sample manipulations and transfer steps, leading to a long overall analysis time [159]. One way two chromatographic methods can be combined on-line is to use a precolumn for the first separation step. The components eluting from this first column are then transferred directly to a second, analytical column for further separation [160]. This on-line combination of columns can be helpful for automated sample pretreatment and untargeted metabolomics [141,160–166]. A major obstacle to the routine implementation of multidimensional chromatographic methods or multiple, distinct column types is throughput when the study size reaches thousands or more samples [99,137–139,150]. Practical considerations such as cost and experience also need to be considered when using multidimensional methods.

4. Types of liquid chromatography (LC) used in metabolomics

Methods in LC are often categorized in terms of the general type of stationary phase employed and the mechanism of separation provided by this stationary phase. As demonstrated by Fig. 4, the two main types of LC used for metabolomics are RPLC and HILIC [97,167,168]. However, other categories of LC have also been used in metabolomics for sample preparation or analysis. These other LC methods include ion-exchange chromatography (IEC), size-exclusion chromatography (SEC), normal-phase liquid chromatography (NPLC, of which HILIC is a subset), adsorption chromatography, and affinity chromatography (AC) [97,98]. In addition, there are some LC methods that combine several of these separation modes within a single column or as part of a multidimensional separation scheme. The following section will examine each of these forms of LC and the ways they have been employed in metabolomics.

4.1. Partition chromatography and related methods

4.1.1. Reversed-phase liquid chromatography (RPLC)

RPLC (also known as reversed-phase chromatography) is the most common form of LC that has been used in metabolomics [97,167–169]. This is demonstrated in Fig. 4 by the number of reports that have used RPLC when compared to other types of LC. RPLC is a type of partition

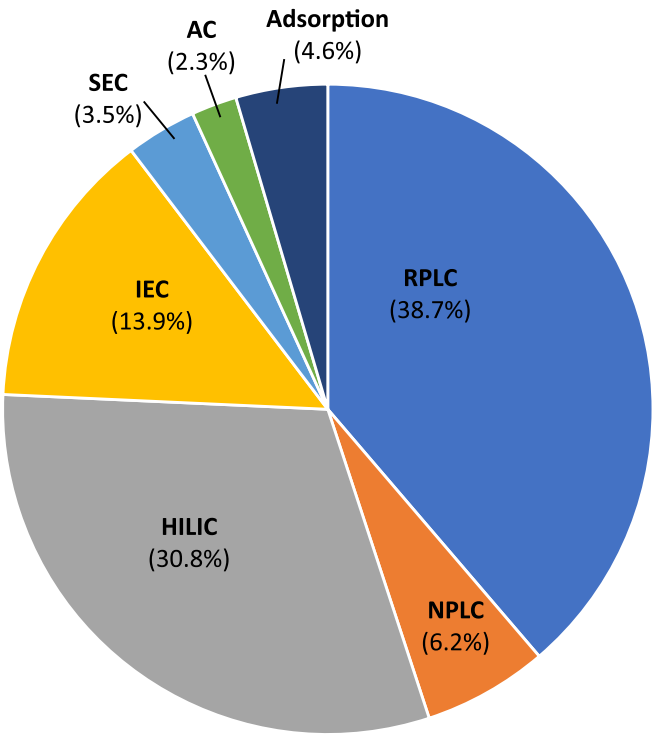

Fig. 4. Relative number of publications containing the term "metabolomics" plus one of the following descriptors for a given type of LC: "reversed-phase chromatography" (RPLC); "HILIC" (hydrophilic interaction liquid chromatography); "normal-phase chromatography" (NPLC), not including HILIC; "size-exclusion chromatography" (SEC); "ion-exchange chromatography" (IEC); "adsorption" chromatography ("silica"-based); and "affinity chromatography" (AC). These results were obtained from searches conducted on the Web of Science in September 2023. There is a total of 2,476 papers that are represented in this plot. The number of papers listed for RPLC are likely a low estimate, as many reports in metabolomics use the more generic term LC-MS when RPLC is used as a separation method with MS.

chromatography that separates compounds based on polarity and by employing a non-polar stationary phase, such as octyl (C_8) or octadecyl (C_{18}) groups [95–97,169,170]. The fact that water is a weak mobile phase in RPLC has made this method particularly appealing for separating and analyzing targets such as metabolites in biological specimens and in aqueous samples [96–98].

RPLC has been used in both targeted and untargeted metabolomics and is often combined with MS [41]. RPLC and RPLC-MS may be done as part of a system that employs either HPLC or UHPLC [41,124–127]. RPLC is a key tool for metabolomics because it works easily with aqueous samples and separates chemicals based on the general property of polarity [97,98]. A few examples of the many classes of metabolites that have been resolved and examined by RPLC include amino acids, carbohydrates, lipids, organic acids, nucleotides, peptides, and vitamins [97]. Specific examples of compounds that have been separated by RPLC for metabolomics include triglycerides, cholesterol esters, sphingomyelins, phosphatidylcholines, diacylglycerols, phosphatidylethanolamines, fatty acids, and eicosanoid [79]. Metabolites and compounds with low polarity will show the highest retention. In contrast, compounds with high polarity (*e.g.,* sugars and amino acids) may be only weakly retained and even require derivatization to increase their retention time on a column for RPLC [97].

RPLC has also been used in metabolomics for sample pretreatment. Examples are the use of C_{18} columns or pre-columns for the extraction of steroids and neurosteroids from several types of biological samples [171–173] and the isolation of various classes of metabolites from urine [174,175]. For instance, a C_{18} column has been used to extract and

preconcentrate metabolites produced by *Lactobacillus sakei* in growth media [176]. Multiple RPLC columns (C_8 and C_{18}) have also been combined with LC-MS to extract metabolites such as amino acids, phospholipids, carnitines, bile acids, free fatty acids, and sphingomyelins from plasma and liver tissue [177]. In addition, RPLC has been often used directly in combination with many other types of LC in multidimensional separations for metabolomics. Examples that will be described in more detail in the following sections are the use of RPLC with HILIC and other forms of LC [120–122,142,151–157,178].

4.1.2. *Normal-phase liquid chromatography (NPLC) and HILIC*

Another type of partition chromatography is normal-phase liquid chromatography (NPLC, also known as normal-phase chromatography) [96–98,169]. NPLC differs from RPLC by using a polar stationary phase, such as diol, cyano, or aminopropyl groups [96–98]; thus, NPLC will have its strongest retention for polar compounds. NPLC is not as common as RPLC in metabolomics, clinical analysis, and related fields because NPLC requires sample components to be injected in a nonpolar solvent [98]. However, normal-phase columns are useful when dealing with compounds that may be present in an organic solvent (*e.g.,* through liquid–liquid extraction) and/or that contain one or more polar functional groups. Some examples include lipids, steroids, and sugars [98,173]. For example, NPLC has been used to fractionate various classes of lipids in rat brain tissue and to separate lipoidal steroids [173].

HILIC is another type of partition chromatography that uses a polar stationary phase [97,167,179]. After RPLC, HILIC is the second most common type of LC that is utilized in metabolomics (see Fig. 4). This method separates chemicals based on their ability to disperse between an organic-rich region of the mobile phase and a more polar water-enriched layer that is located at or near the surface of a polar support. HILIC often begins with a mobile phase that contains a small amount of water (*e.g.,* 5 %) in an organic solvent such as acetonitrile to form the water-enriched layer. The water content of the mobile phase is then gradually increased to promote elution [97,180]. The surface of the support in HILIC may contain a polar, noncharged surface (*e.g.,* silanol groups on unmodified silica) or surfaces that have been modified to contain diol, amide, or cyano groups [97,181]. Charged groups may also be present, as occurs in the use of supports that have surfaces with protonated amines, carboxylates, or zwitterionic groups (*e.g.,* with the latter being referred to as zwitterionic HILIC or ZIC-HILIC) [97].

HILIC has been used in metabolomics and other areas as a tool to retain and separate polar and charged compounds found in biological samples [167,182,183]. Compounds in this group range from small organic acids and amino acids to nucleosides, sugars, and phosphate-containing compounds, as illustrated in Fig. 5. The ability of HILIC to retain and separate these polar compounds makes this method complementary to RPLC, which instead has its highest retention for nonpolar analytes [184–187]. One way HILIC has been used in metabolomics is as a pretreatment method for polar metabolites [7,188]. This has included the use of HILIC cartridges to extract phenolic metabolites from plasma and urine samples [188] and the use of a HILIC column to enrich hydrophilic metabolites and amino acids in blood for analysis by targeted metabolomics [7].

Many studies have also used HILIC in analytical separations for metabolomics. Like RPLC, HILIC is often used with MS for this purpose [69,152,189]. The fact that HILIC has multiple modes of interaction can make this method more versatile than RPLC and potentially allow for improved metabolite coverage [189,190]. However, the same feature makes it more difficult to use HILIC with regard to predicting analyte retention [191]; this factor has led to HILIC being used in metabolomics mainly for untargeted studies [189,190]. HILIC has been used with MS to simultaneously analyze short-, medium-, and long-chain aromatic amines and acylcarnitines that are responsible for insulin resistance in type 2 diabetes in several human population studies [192,193]. A method to examine acylcarnitine and amino acid metabolism was developed based on HILIC combined with MS and used to profile

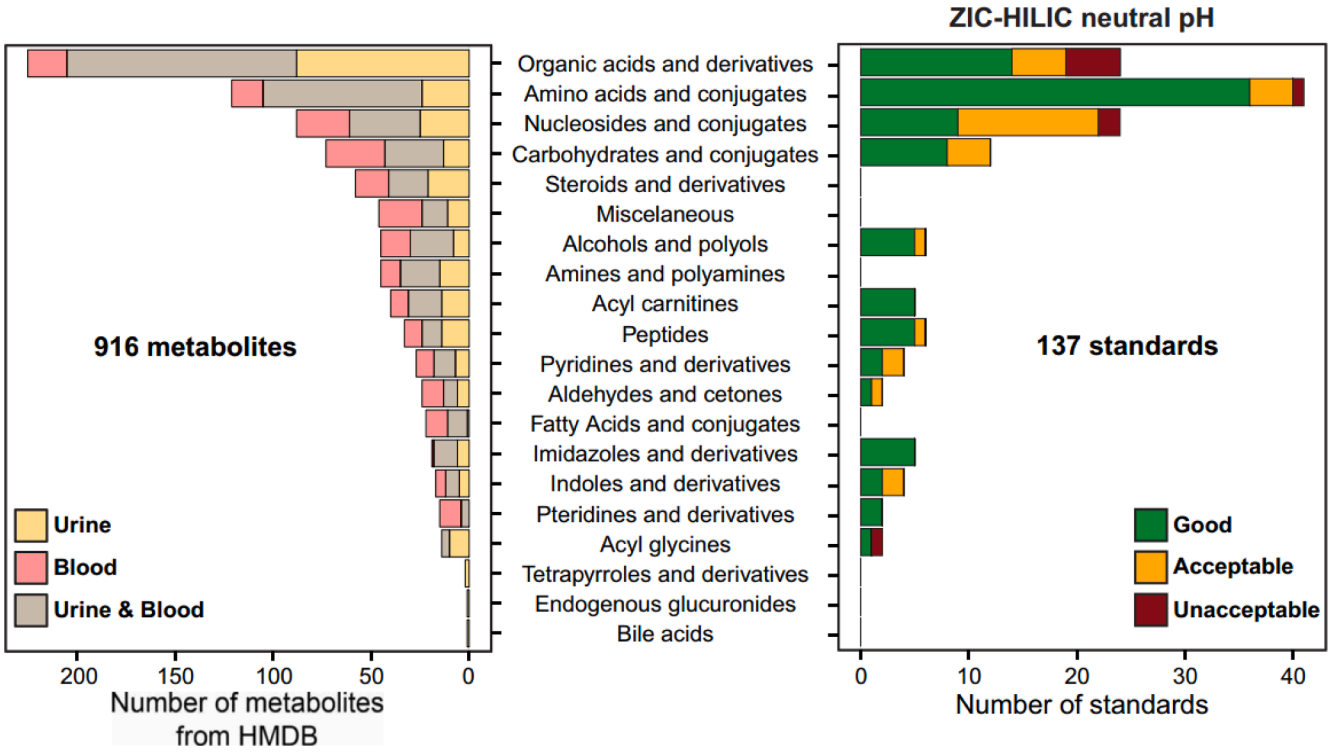

Fig. 5. Examples of compounds that are suitable for analysis in untargeted metabolomics by HILIC-MS when working with urine and plasma, as illustrated for an optimized separation method using zwitterionic HILIC (ZIC-HILIC) at a neutral pH. The graph on the left shows the number of metabolites of various classes that are listed in the Human Metabolome Database (HDMB) as having been detected and quantitated in urine and blood. The graph on the right shows the results obtained with ZIC-HILIC for representative standards from each of these compound classes. This figure is reproduced from Ref. [152] with permission (Elsevier).

samples such as plasma, urine, cerebrospinal fluid, and tissue extracts [194].

A growing number of researchers are using HILIC in combination with RPLC and MS [120–122,151–158]. For instance, this combination of techniques has been employed to separate and resolve over 150 metabolites in rice [151]. This set of tools has also been used together in the non-targeted metabolomics analysis of other food products [156]. The use of HILIC plus RPLC with MS was found to increase the coverage of the metabolome in urine and plasma samples by approximately 44 % and 108 %, respectively, when compared to the use of RPLC along with MS [152]. UPLC and MS have been used with both RPLC and HILIC for untargeted metabolomics studies to identify potential biomarkers for liver cancer or breast cancer [121,153].

Despite the complementary nature of RPLC and HILIC, many metabolomics studies employing LC with MS still use a single type of column. This is believed to be linked to the increase in time and cost when using multiple columns. RPLC is also more commonly used with MS than HILIC in metabolomics [190,195,196]. This is partly due to RPLC being a more established method with a long history and high degree of familiarity in clinical analysis and biomedical research [96–98]. However, part of the preference for RPLC may be linked to the perception that RPLC-MS provides better performance than HILIC-MS and is a more robust analytical approach in areas such as metabolomics [152,197–199]. In addition, HILIC typically produces broader

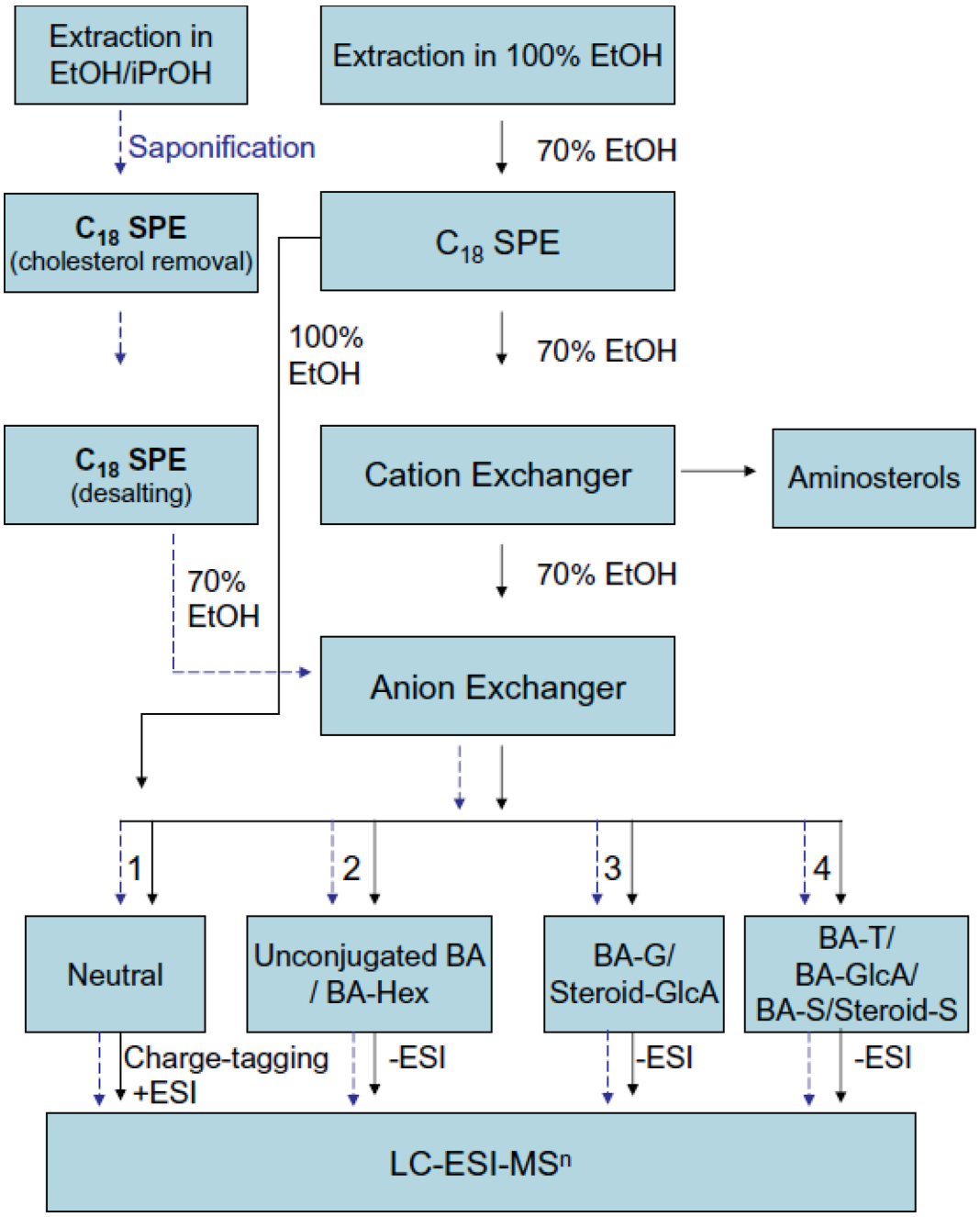

Fig. 6. Strategy for the pretreatment of neutral and acidic steroids/bile acids prior to their LC-MS with electrospray ionization (ESI). This scheme uses a combination of extraction with ethanol (EtOH) or an ethanol/isopropanol (EtOH/iPrOH) mixture followed by C_{18} reversed-phase solid-phase extraction (C_{18} SPE) and the use of cation-exchange chromatography or anion-exchange chromatography. Other abbreviations: BA, bile acid; BA-G, glycine conjugated bile acid; BA-GlcA, glucuronic acid conjugated bile acid; BA-Hex, hexose conjugated bile acid; BA-S, sulfated bile acid; BA-T, taurine conjugated bile acid; Steroid-GlcA, neutral steroid conjugated with glucuronic acid; and Steroid-S, sulfated neutral steroid. This figure is reproduced from Ref. [200] with permission (Elsevier).

peaks than RPLC, resulting in a reduced peak capacity and greater reliance on the resolution capabilities of the mass spectrometer in HILIC [180,182].

4.2. Ion-exchange chromatography (IEC) and related methods

RPLC works well for many nonpolar metabolites or metabolites with medium polarity. However, this separation method may be difficult to use with solutes that contain a charge. In this situation, an alternative is to use IEC [96–98]. IEC is a form of LC in which a support containing fixed charges on its surface is used to bind and separate solutes with complementary charges. IEC can be divided into two categories based on the type of charge present on the support and stationary phase. In cation-exchange chromatography, the stationary phase consists of negatively-charged groups that are used to retain positive ions. In anion-exchange chromatography, the stationary phase has positively-charged groups that are used to bind and separate negative ions [96–98,169].

Both cation- and anion-exchange chromatography have been used for sample pretreatment and fractionation prior to the analysis of metabolites by other LC methods combined with MS [167]. For example, strong cation-exchange supports have been used to extract more than 140 charged and water-soluble metabolites from cultures of *Escherichia coli* and *Saccharomyces cerevisiae* prior to a further separation and analysis by HILIC and MS [142]. In another report, both anion- and cation-exchange chromatography were used to fractionate components of the steroid metabolome (*e.g.,* bile acids, oxysterols, and hormonal steroids) before these compounds were separated and analyzed further by LC-MS (see Fig. 6) [200].

IEC has been directly used in other studies in the specialized form of ion chromatography (IC) for analytical separations and in combination with detectors based on conductivity, fluorescence, amperometry, or MS [97,98,167]. The need to address the salt content and relative volatility of the mobile phase contents in IEC is an issue that must always be considered when combining this method with MS [201–203]. For instance, the coupling of IC with MS requires the use of an ion-exchange column with an on-line electrolytic eluent suppressor system to convert a mobile phase that may have a high concentration of competing ions to an aqueous solution that contains little or no competing ions [96,97]. An organic solvent can also be added to the mobile phase through a mixing tee to provide an eluent that is more compatible with electrospray ionization for MS [167].

IC in the form of capillary IC-MS has been used as the basis for several metabolomics studies. Capillary IC-MS with a strong anion-exchange medium has been employed to analyze organic acids and sugar di/tri-phosphates from the protozoan *Trypanosoma brucei* [204] and to examine metabolites related to carbon metabolism in glioblastoma cells [205]. This combination of methods has further been used to profile agents such as sugar phosphates and nucleotides in extracts of human kidney HEK293 cells [206] and to examine nucleotides, organic acids, sugars, and sugar phosphates in SW480 cancer cells [207]. IC-MS with anion-exchange has been used to evaluate phosphorylated metabolites after their enrichment using metal oxide affinity chromatography [208]. A comparison has also been made of LC-MS methods based on RPLC, HILIC, or anion-exchange LC in terms of their detection and coverage of 354 metabolites from 19 compound classes in mammalian samples [178]. HILIC and RPLC gave retention factors greater than one for 54 % and 41 % of these metabolites, respectively. Combining HILIC with RPLC increased the overall metabolite coverage to 92 %, and including anion-exchange chromatography (*e.g.,* for short chain fatty acids) further increased the coverage to 97 % [178].

Several reports have considered the direct coupling of IEC with other forms of LC for multidimensional separations. RPLC and anion-exchange chromatography have been used with MS for metabolomic analysis as related to environmental exposure (*i.e.,* the exposome) [209]. A scheme based on RPLC followed by IEC and MS was used to detect over 80 metabolites in plasma and urine samples [210]. HILIC has been used in a mixed-mode format with weak anion-exchange chromatography and MS to determine glyphosate and its metabolite aminomethylphosphonic acid in fruits and vegetables [211]. Strong cation-exchange chromatography was used with RPLC and a chip-based format for the analysis of metabolites and peptides in spleen samples [212].

Another way in which a separation based on ion-exchange can be combined with a second separation mode is through the method of ion-pair chromatography (IPC), which is also referred to as ion-pair liquid chromatography (IPLC) [28,66,97]. IPC typically uses a column for RPLC and a mobile phase that contains an ion-paring agent with a non-polar tail (*e.g.*, for interacting with a RPLC column) and a charged section for interacting with an analyte that contains the opposite charge [97,213,214]. Examples of ion-pairing agents in IPLC are perchlorate or alkane-sulphonic acids (*e.g.,* as occurs in sodium dodecyl sulfate) for the separation of cations and tetrabutylammonium or related surfactants for the separation of anions [95,213].

Several reports have used IPC for metabolomics, either alone or in combination with other LC separation methods. One study compared the use of HILIC-MS, RPLC-MS, and IPC-MS for the analysis of polar and/or ionic metabolites, with a particular emphasis on compounds related to central carbon metabolism [214]. Of these three methods, only IPC-MS was found to provide a sufficiently robust method (when suitable precautions and conditions were followed), making it possible to profile over a hundred organic metabolites in serum, urine, and tissue extracts [214]. IPC-MS was used to detect small polar molecules, such as through the use of diamyl ammonium acetate as an ion-pairing reagent for the retention of acidic polar compounds [215]. A C_{18} based column was used either directly or with IPC to give separations based on reversed-phase, cation-exchange, anion-exchange, and normal-phase modes for the separation of polar metabolites [80].

4.3. Adsorption liquid chromatography

Another type of LC based on stationary phase and separation mode is adsorption chromatography. In this method, solutes are separated based on their adsorption to the surface of a support [96–98,171,173]. Adsorption chromatography is not usually used for the analytical separation of biological samples for metabolomics but is instead employed for sample pretreatment. Supports that are frequently used for sample pretreatment by adsorption chromatography and SPE include polymeric supports (*e.g.,* polystyrene) and non-derivatized silica [96]. Similar supports for adsorption chromatography are also often used in planar chromatography [97,98]. Examples of adsorption chromatography in metabolomics include the use of polymeric resins to extract steroids and neurosteroids [171,173]. In addition, silica on TLC plates has been used for the separation of lipids in brain tissues and their analysis by MS [103].

4.4. Size-exclusion chromatography (SEC) and related methods

Separation of compounds based on their size/shape and ability to partition into a porous support forms the basis for SEC [96–98]. Although most of the compounds examined in metabolomics have relatively small masses and cannot be separated from one another by SEC, this method is still important in removing proteins and other higher-mass substances from smaller metabolites [97]. For instance, the removal of proteins in metabolomics is often required to avoid matrix effects and ionization suppression in the use of LC with MS [35,46,78–80]. The retention of biomolecules in a metabolomics sample has been shown to non-uniformly perturb metabolite concentrations primarily through protein binding interactions [58,216,217]. Protein precipitation with an extraction solvent (*e.g.,* methanol) is a preferred approach to remove biomolecules and release the bound metabolites [217]. While ultrafiltration is also used to remove biomolecules, this technique may selectively deplete protein-bound metabolites from a metabolomics analysis based on MS or NMR. Sample preparation by SEC

is often carried out using low-performance size-exclusion media in solid-phase extraction cartridges or spin columns. Alternatively, SEC can be employed to isolate a large biological agent that may contain bound or encapsulated metabolites. Examples of this latter application are the use of SEC for the isolation of metabolites that are associated with proteins or extracellular vesicles [218–222].

Alternatively, SEC has been used in metabolomics to specifically analyze and characterize the interactions of metabolites with proteins and other biomacromolecules [4,219,223–225]. In this type of work, SEC can be used to separate free metabolites and metabolite–protein complexes based on their large differences in size. One recent study used SEC to examine metabolite-protein interactions in cell-free lysates from cultures of the plant *Arabidopsis thaliana* [219]. SEC may also be used with known mixtures of a metabolite and a protein to separate and measure the free and bound fractions of a metabolite; this information can then be used in binding studies to provide information on the equilibrium constant for the metabolite–protein interaction [4,223–225].

Another way size-based separations can be utilized in metabolomics is through the use of restricted access media (RAM) [162,226–230]. This type of material consists of a porous support in which only the interior contains the desired stationary phase, such as non-polar groups or ion-exchange sites [78,231–237]. The pore size is set so that only small compounds such as metabolites are allowed to enter the interior region while prohibiting the entry of larger agents such as proteins. This ability makes RAM columns useful in on-line sample pretreatment for work in areas such as untargeted metabolomics [162,226–230].

Turbulent flow chromatography (TFC) is an additional way in which size-based separations can be combined with other forms of LC for use in metabolomics. TFC is also known as ultra-high flow or high-flow chromatography [89,238–240]. TFC often makes use of a relatively large diameter RPLC support (*e.g.*, 25–50 μm) that is operated at a high flow rate (*e.g.*, 1.5–5.0 mL/min). These conditions can generate turbulent flow within the column that enables macromolecules such as proteins to be readily washed away, while allowing smaller molecules to enter the pores of the particles and to be retained for further separation and analysis [240,241]. A common application for this method is to remove proteins and to isolate drug metabolites for the analysis of complex matrices such as urine, plasma, tissue homogenates, cerebrospinal fluid, and environmental samples [242–247].

4.5. *Affinity chromatography (AC) and related methods*

Another type of LC is affinity chromatography (AC) [72,96,248]. This method is based on the selective and reversible interactions between biomolecules [72,248]. Immunoaffinity chromatography (IAC) is a subset of AC in which an antibody or related binding agent is employed [72,97,249,250]. The high selectivity and strong binding of antibodies for their targets have made IAC an important tool for sample pretreatment and the isolation of specific sample components for applications related to targeted metabolomics [72]. This form of IAC is often referred to as immunoextraction and can be used either off-line or directly with other analytical techniques [250]. This has included the on-line use of IAC with further LC separation and MS for the trace analysis of specific compounds and their metabolites in matrices such as food, plant or animal tissues, clinical specimens, and environmental samples [72,249–263].

IAC has been used in several studies to isolate mycotoxins and related metabolites for the measurement or characterization of these components [251–254]. For instance, IAC has been used to enrich and detect deoxynivalenol and fungal-derived metabolites from grains [254]. An IAC column has been employed to isolate and aid in the detection of residues and metabolites related to the steroids methandrostenolone and brassinosteroid [255,258]. Aflatoxins, ochratoxins, and related metabolites have been isolated from samples such as cocoa, oil, spices, cereals, nuts, and infant formula by using immunoaffinity columns [256,257]. Broad-specificity monoclonal antibodies were used to purify and enrich brassinosteroid metabolites from minute plant samples before further analysis [258]. IAC has been coupled on-line with RPLC for the trace analysis of triazine herbicides and their metabolites or degradation products [264–267]. In addition, IAC has been used on-line with LC-MS for trace analysis of LSD and its metabolites in samples such as urine or plasma [259,260].

Other binding agents have also been used in AC for metabolomics. For example, ergosterol biosynthetic enzymes were tagged with an immunoglobulin G-binding domain [268]. Binding by metabolites to these enzymes was then examined after the affinity-based isolation of the tagged proteins [268]. Metal oxide AC based on zirconia or titania was used to enrich phosphorylated metabolites prior to their separation and analysis by IC-MS [208]. The use of traditional immobilized metal-ion AC for sample pretreatment was considered in the same report [208].

AC and high-performance affinity chromatography (HPAC) have been used to study interactions by proteins and other biomacromolecules with metabolites of drugs and hormones [4,224,225,269–271]. Examples include the application of HPAC to compare and characterize the binding of thyroxine, triiodothyronine, and related metabolites with human serum albmin (HSA) [272,273]; the interactions of phenytoin and its major metabolites with HSA [274,275]; and the binding of warfarin and L-tryptophan and related coumarin or indole compounds at Sudlow sites I or II of HSA [276,277]. HPAC has also been used to determine rate constants for interactions of the individual chiral forms of phenytoin metabolites with HSA [278].

5. Gas chromatography (GC) in metabolomics

GC is a chromatographic method in which the mobile phase is a gas and sample components are separated in the gas phase [96–98]. Although LC is more commonly used in metabolomics, GC is also an important separation tool in this field [279–316]. This is especially true when the goal is to work with volatile compounds or compounds that can be readily converted into a volatile form [97,280,281,304,305]. The relative extent of use for GC vs LC in this area is illustrated in Fig. 7 by the number of papers that have appeared between 2000 and 2022 that have contained the terms “metabolomics” plus “gas chromatography” vs “liquid chromatography”. In this comparison, GC was used in about one third of the reports over 2000–2022 that used either LC or GC for metabolomics. In addition, the use of GC to analyze metabolites goes back many decades and predates the modern era of metabolomics. For instance, one of the first applications of GC occurred in the early 1950’s when it was used to separate methyl esters of fatty acids [97,280]. GC is most often used in metabolomics in the form of GC–MS [279,281,282,304], although multidimensional GC–MS (*e.g.,* 2D GC–MS) has also been used for untargeted and targeted metabolomics [147–149,305]. Examples of metabolite classes that have been analyzed by GC–MS include amino acids, organic acids, catecholamines, fatty acids, hormones, hydroxyl acids, sterols, and sugars, among others [281,305].

Advantages of GC–MS are its high efficiency and good resolution for volatile compounds and the availability of well-established libraries of mass spectra that can be used for compound identification [97,281,282,304]. One limitation of GC–MS is the requirement that its analytes be both sufficiently volatile and thermally stable at the temperatures that are employed for sample injection and elution [97,98,305]. Meeting this requirement often means that analytes must first be derivatized to convert them into a volatile and thermally-stable form (*e.g.*, through the use of silylation or the formation of methyl esters) [57,97,98,283–288,304,305]. This form of pretreatment is important in metabolomics because it significantly expands the range of compounds that can be examined by GC–MS [304,305]. Fig. 8 gives a typical workflow for GC–MS in metabolomics that includes a derivatization step [304].

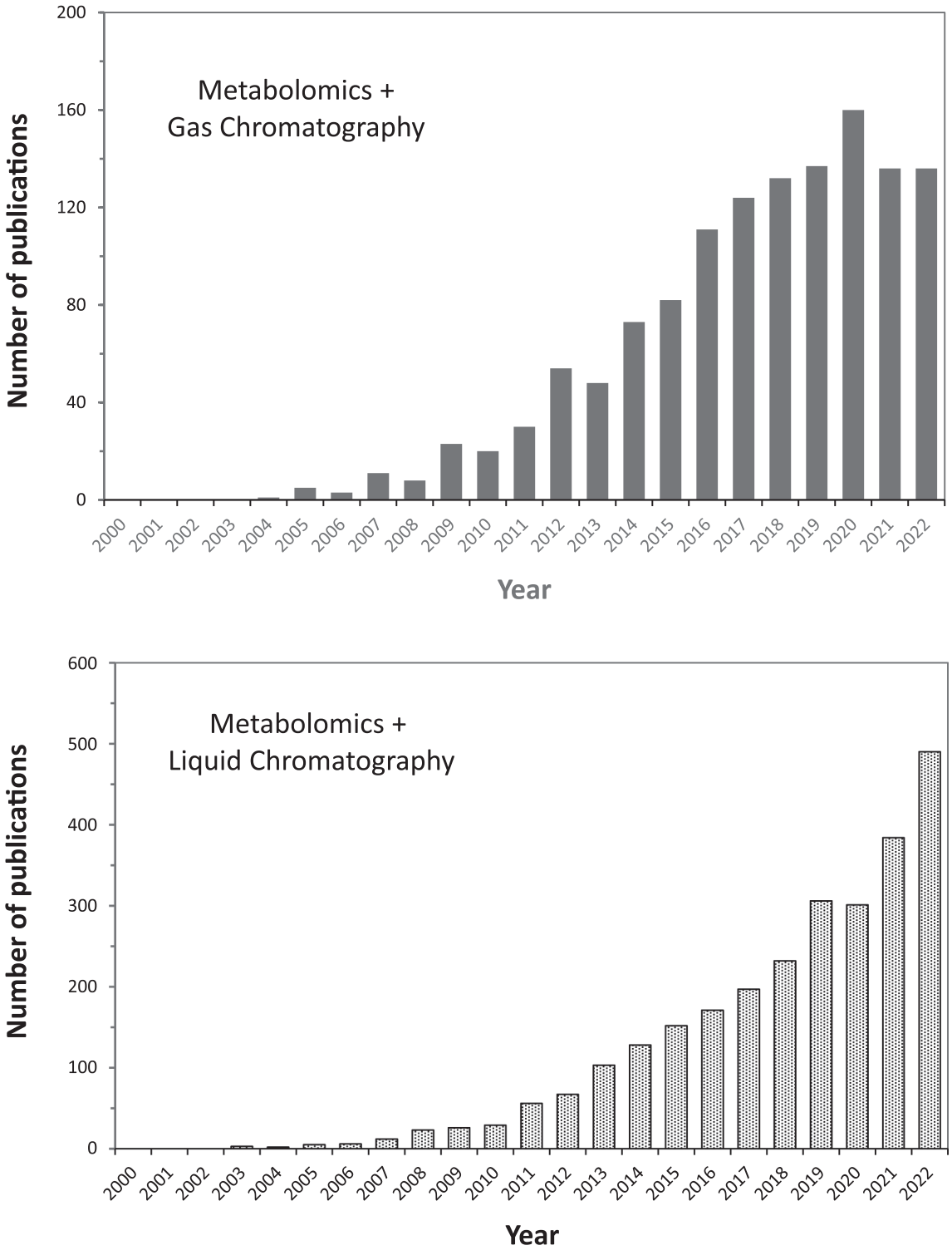

Fig. 7. Number of publications which included the terms " liquid chromatography" or "gas chromatography" plus "metabolomics" and which were published between 2000 and 2022. These results were obtained from searches conducted on the Web of Science in February 2024. These numbers are probably low estimates of the total papers in each of these areas, as some reports that use liquid chromatography or gas chromatography may use the abbreviations "LC" or "GC" rather than the full names of these techniques.

Two fields in which GC–MS has been used for metabolomics are clinical analysis and biomedical research [279,291,298–313]. For instance, GC–MS has been used to analyze metabolites in human plasma [298] and for profiling of endogenous agents such as organic acids, amino acids, and sugars in urine, serum, or plasma [291]. The use of GC–MS in the study of cancer is another important application in the field of metabolomics [306–310]. For instance, GC–MS has been used in metabolomics to compare hepatocellular carcinoma with healthy surrounding tissue [306] and to examine metabolites in plasma for patients with medullary thyroid cancer vs the plasma of healthy individuals [307]. GC–MS has been used to identify metabolites in urine that may be used for the diagnosis of non-Hodgkin's lymphoma [309] and to

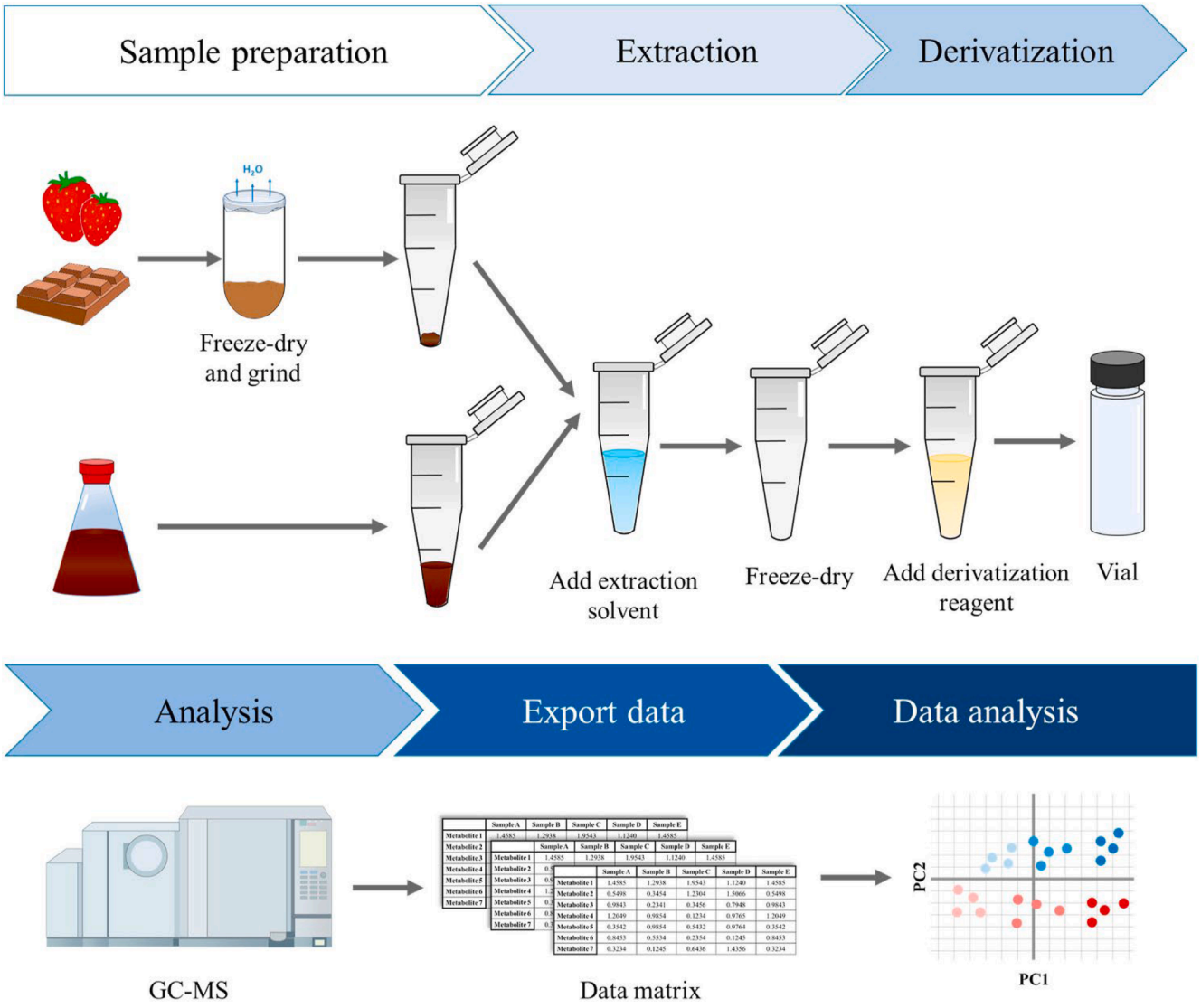

Fig. 8. Example of a general workflow for the preparation and analysis by GC–MS for samples in metabolomics. The derivatization step on the right is needed for work with non-volatile or thermally-unstable compounds. This figure is reproduced from Ref. [304] with permission (Elsevier).

compare healthy vs cancerous prostate epithelial cells [310]. A 2D GC method has been employed with untargeted metabolomics to analyze the contents of serum from patients with colorectal cancer [308]. Both GC–MS and 2D GC–MS have also been used in metabolomics studies involving yeast and microbial systems, as well as in other biomedical applications [287,311,312].

GC–MS has been frequently employed to examine both volatile metabolites and low mass non-volatile metabolites (*e.g.,* through derivatization) in plants and food-related matrices [289–297,304,311–316]. One report used GC–MS for plant metabolomics to examine changes in sugars, amino acids, organic acids, and some inorganic acids that were present in radish roots stressed by exposure to lead or cadmium [290]. Applications in food science have included the metabolic profiling, quality analysis, and authentication of foods, along with studies of the growth, processing, stressing, and storage of foods [304]. For instance, GC–MS has been employed to investigate flavor development in shrimp paste [313]. Metabolomics based on both GC and LC have been used to study the volatile plus nonvolatile compounds involved in the withering process of black tea [314]. GC–MS and metabolic profiles have been used to characterize the regions in which certain foods or their components have originated, such as has been used for some varieties of rice or whole grain liquor [315,316].

6. Supercritical fluid chromatography (SFC) in metabolomics

SFC is a form of chromatography in which a supercritical fluid is the mobile phase [96–98,317–323]. SFC has not been used as extensively as LC or GC in metabolomics, with the number of papers in this area being about 1 % of those shown in Fig. 7. However, there has been increasing and ongoing interest in the unique capabilities SFC may offer for metabolomics [317,318,321,322]. For instance, a supercritical fluid has a lower viscosity than a liquid, along with a higher density than a gas [97,98,317,318]. These properties mean SFC will have retention behavior and efficiencies that are between those of LC and GC. Furthermore, SFC can often be used with modified forms of equipment, stationary phases, and supports that were originally created for either GC or LC [97,98], including the use of both HPLC and UHPLC columns [124,318–323]. Stationary phases for SFC have included those developed for RPLC, NPLC, and HILIC [97,318–323].

SFC has been combined with MS for various applications in metabolomics. As an example, SFC-MS has been used for comprehensive lipid profiling and the separation of polar metabolites [317,321,322]. The use of SFC-MS in pharmaceutical analysis has been considered [318], and the use of SFC-MS with UHPLC-type columns and various stationary phases has been evaluated for the analysis of medium and high polarity

metabolites in urine [323]. SFC-MS using UHPLC columns has been demonstrated to separate both polar and non-polar lipids [322]. Along with the possible addition of methanol to the mobile phase, it has been found that ionizable compounds such as poly acids, aliphatic amines, and other polar metabolites can be separated by SFC when utilizing small amounts of acids, bases, or salts as mobile phase modifiers [317,318]. A small amount of water can also be used with CO_2 and methanol to elute highly polar compounds and metabolites in SFC [317,318].

7. Chromatography in NMR-based metabolomics

Many of the prior examples given in this review have involved the combination of LC, GC, or SFC with MS for metabolomics. This reflects the fact that LC-MS is currently the most common approach used to analyze samples for metabolomics, with GC–MS also playing an important and growing role in this field [289–305,324]. In each of these methods, a separation based on some form of chromatography is generally required prior to MS to resolve the many compounds that occur over the relatively narrow molar mass range of the metabolome [70]. This separation step can further be employed to minimize effects by the matrix or co-eluting compounds that may lead to ion suppression in MS [35,45,46,78–80,325]. However, neither the presence of metabolites over a narrow mass range nor ion suppression are issues in NMR, although sample matrix effects due to proteins or lipids may still occur [6,58,90–92]. Thus, chromatography has not been as frequently used in NMR as it has been in MS for metabolomics, as shown earlier in Fig. 2.

There are several other factors that have led to chromatography not being part of many prior NMR-based studies. One primary reason is it is desirable to minimize sample handling and the overall time it takes to acquire an NMR spectrum for a sample. This is required because metabolites are readily interconverted due to residual enzymatic activity or oxidation and may be subject to such issues as thermodynamic instability or degradation and solubility [85–88,326,327]. Another concern is the change in relative concentration that may occur for a metabolite during pretreatment [328]. Compound decomposition during the separation process, inefficient or non-uniform compound derivatization, and less than 100 % compound recovery are other ways chromatography may impact the outcome of metabolomics studies, as seen in LC-MS [328–330]. In addition, the impact of these factors may not be uniform for all metabolites and may give an incorrect impression of the relevance of a given change on the system under investigation. For example, such effects may lead to a metabolite being incorrectly identified as a potential biomarker for cancer [331].

NMR does face challenges in metabolomics with peak overlap, low sensitivity, and a limited dynamic range; however, these issues can be partly overcome by employing multidimensional NMR experiments instead of chromatography. For instance, NMR methods such 2D ^{1}H–^{13}C heteronuclear single quantum coherence spectroscopy (HSQC), heteronuclear multiple bond correlation (HMBC), ^{1}H–^{1}H total correlation spectroscopy (TOCSY), and HSQC-TOCSY are frequently utilized in metabolomics to assist with metabolite annotations and can be used in combination with LC-MS [6,54–56,332–336]. Analogous schemes based on MS/MS are used in metabolomics [337,338], although these methods are not replacements for chromatography in the same way as when NMR peaks are spread across two dimensions using data based on ^{1}H and other nuclei (*e.g.,* ^{13}C, ^{15}N, or ^{31}P) [339,340]. In addition, non-uniform sampling, rapid acquisition methods and super sequences based on NMR by ordered acquisition using ^{1}H detection can be used for rapid (<ms) 2D NMR collection for metabolomics [56,341–345].

Despite the differences in the need for chromatography in NMR and MS, there is a growing interest in combining LC with NMR for metabolomics. This is demonstrated in Fig. 2 by the increasing number of publications in NMR-based metabolomics that have employed chromatography. The main reason for this interest is the same as it is for MS, in that using LC with NMR can simplify complex heterogeneous mixtures before their analysis [346–349]. In principle, all the chromatographic techniques discussed in Section 4 can be employed with NMR in either an on-line or off-line mode. The easiest of these two modes is to use off-line coupling of LC with NMR, as illustrated in Fig. 9 for a scheme that couples RPLC with ^{1}H NMR in metabolomics [347]. The use of on-line LC with NMR requires interfacing LC with an NMR flow probe [350,351] to rapidly transfer sample aliquots into a stationary chamber positioned inside the magnet of the NMR spectrometer. In either off-line or on-line modes, the metabolomics samples will need to be placed into an NMR-friendly solvent that includes a deuterated buffer and has a low salt concentration (*i.e.,* < 50–100 mM for use with cryoprobes) [352].

8. Conclusions

This review examined the use of chromatography and its various forms in the field of metabolomics. This included the use of chromatography with either MS or NMR and the use of LC, GC, or SFC for the pretreatment or separation of compounds in samples for metabolomics. General considerations for sample pretreatment and separations in metabolomics were discussed along with the various supports and separation formats for chromatography that have been used in metabolomics. Many types of LC methods were considered with regard to their applications and possible advantages or disadvantages in metabolomics. These methods included RPLC and HILIC, which are the two forms of LC used the most in metabolomics. Other LC methods (*e.g.,* IPC, NPLC, IEC, SEC, AC, and adsorption chromatography) were also discussed that have been used in fewer applications for metabolomics but offer unique abilities that may be valuable for future work in this field. The combination of these techniques in multidimensional LC for metabolomics was further considered, as well as applications of GC and SFC in this area.

Several trends and areas for possible future development were noted in this review. For example, there remains room for significant growth in the use of LC methods other than RPLC or HILIC and in the continued use of GC or SFC with MS for metabolomics. The same is true for NMR-based metabolomics, which has usually been conducted without the use of chromatography in most previous studies and for which the inclusion of chromatography may allow for the analysis of even more complex samples and sets of metabolites. The need for methods that can examine a greater range of metabolites will also probably involve the use of more forms of multidimensional separations with either MS or NMR and combining RPLC and HILIC to a great degree with other forms of chromatography. Finally, little work has been done up to the present in using chromatography with microfluidics for metabolomics. This is another area expected to see future development, as microfluidics has recently been employed for single cell metabolomics [353–356] and in other fields for LC separations or for sample analysis by MS [357–359]. Developments in any of these areas should continue to increase the capabilities and impact that chromatography offers for new research and applications in metabolomics.

CRediT authorship contribution statement

Susan T. Ovbude: Conceptualization, Writing – original draft, Writing – review & editing. **Sadia Sharmeen:** Conceptualization, Writing – original draft, Writing – review & editing. **Isaac Kyei:** Formal analysis, Writing – original draft, Writing – review & editing. **Harshana Olupathage:** Writing – original draft, Writing – review & editing. **Jacob Jones:** Writing – original draft, Writing – review & editing. **Richard J. Bell:** Writing – original draft, Writing – review & editing. **Robert Powers:** Writing – original draft, Writing – review & editing. **David S. Hage:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing.

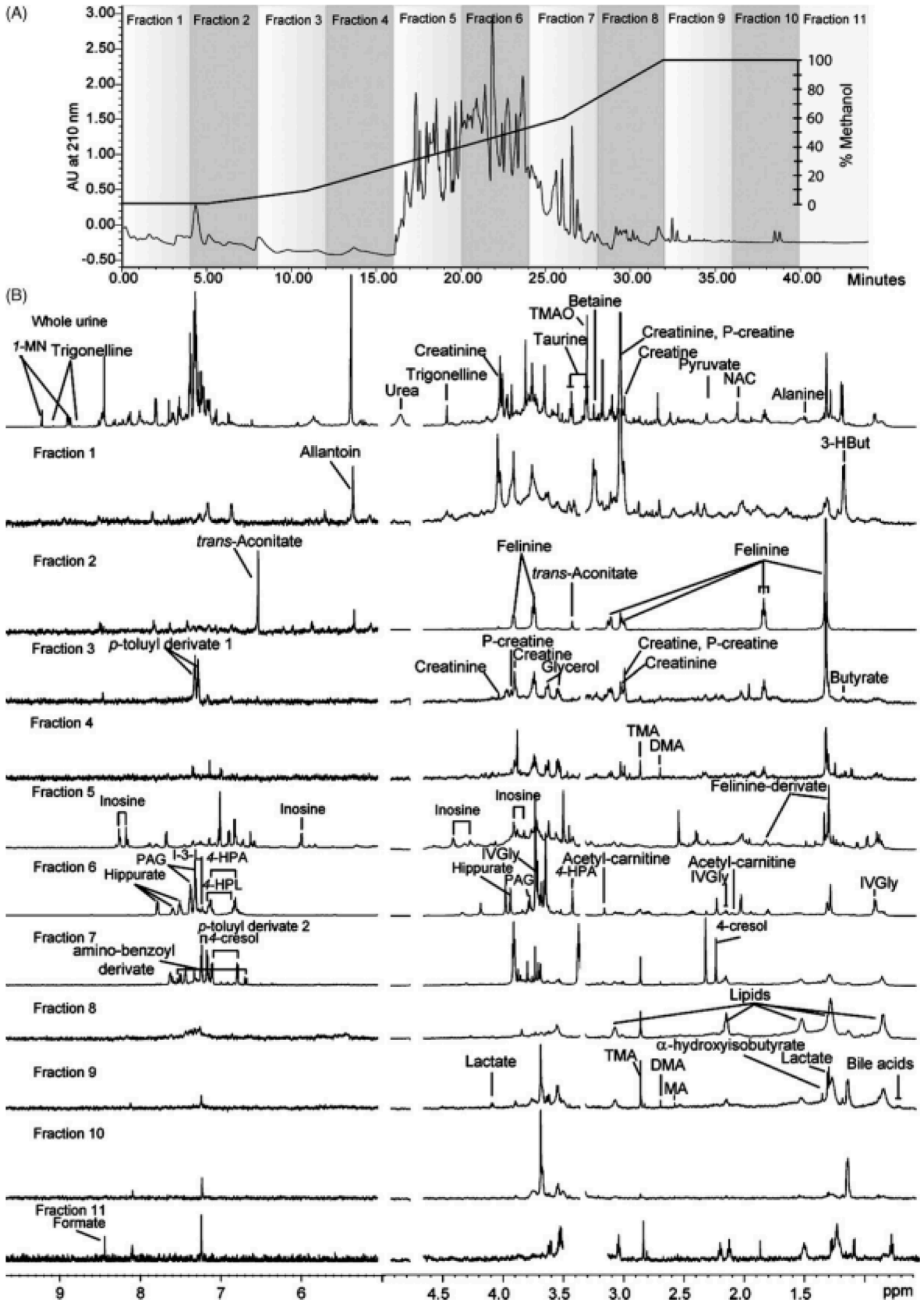

Fig. 9. Use of reversed-phase solid-phase extraction to fractionate cat urine prior to analysis of the fractions by ^{1}H NMR spectroscopy. The chromatogram that was obtained by RPLC for the original sample is shown in (a); the ^{1}H NMR spectra of the collected fractions are provided in (b). Abbreviations: 4-cresol, 4-cresol derivative; DMA, dimethylamine; 3-Hbut, 3-d-hydroxybutyrate; 4-HPA, 4-hydroxyphenylacetate; 4-HPL, 4-hydroxyphenyllactate; I-3-L, indole-3-lactate; IVGly, isovalerylglycine; MA, methylamine; 1-MN, 1-methylnicotinamide; NAC, N-acetyl-glycoproteins; PAG, phenylacetylglycine; P-creatine, phosphocreatine, TMA, trimethylamine; and TMAO, trimethylamine-N-oxide. This figure is adapted from Ref. [347] with permission (Elsevier).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David S. Hage reports financial support was provided by National Science Foundation. Robert Powers reports financial support was provided by National Institutes of Health.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported, in part, by the National Science Foundation under grants CHE 2108881 and CHE 2320239 and by the University of Nebraska Research Council (D.S. Hage). This work was also supported, in part, by the National Institutes of Health (NIH) under grant R01 AI14860, the University of Nebraska Redox Biology Center (P30 GM103335), and the Nebraska Center for Integrated Biomolecular Communication (P20 GM113126) (R. Powers).

References

[1] O. Fiehn, Metabolomics - the link between genotypes and phenotypes, Plant Mol. Biol. 48 (2002) 155–171.
[2] R. Kaddurah-Daouk, B.S. Kristal, R.M. Weishiboum, Metabolomics: a global biochemical approach to drug response and disease, Annu. Rev. Pharmacol. Toxicol. 48 (2008) 653–683.
[3] N.L. Kuehnbaum, P.B. McKibbin, New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era, Chem. Rev. 113 (2013) 2437–2468.
[4] R. Matsuda, C. Bi, J. Anguizola, M. Sobansky, E. Rodriguez, J.V. Badilla, X. Zheng, B. Hage, D.S. Hage, Studies of metabolite-protein interactions: a review, J. Chromatogr. B 966 (2014) 48–58.
[5] P. Miggiels, B. Wouters, G.J.P. van Westen, A.-C. Dubbelman, T. Hankemeir, Novel technologies for metabolomics: more for less, Trends Anal. Chem. 120 (2019) 115323.
[6] J.L. Markley, R. Bruschweiler, A.S. Edison, H.R. Eghbalnia, R. Powers, D. Raftery, D.S. Wishart, The future of NMR-based metabolomics, Curr. Opin. Biotechnol. 43 (2017) 34–40.
[7] L.D. Roberts, A.L. Souza, R.E. Gerszten, C.B. Clish, Targeted metabolomics, Curr. Protoc. Mol. Biol. 1 (2012) 1–24.
[8] L. Cui, H. Lu, Y.H. Lee, Challenges and emergent solutions for LC-MS/MS-based untargeted metabolomics in diseases, Mass Spectrom. Rev. 37 (2018) 772–792.
[9] A.R. Fernie, R.N. Trethewey, A.J. Krotzky, L. Willmitzer, Metabolite profiling: from diagnostics to systems biology, Nat. Rev. Mol. Cell Biol. 5 (2004) 763–769.
[10] H. Tweeddale, L. Notley-McRobb, T. Ferenci, Effect of slow growth on metabolism of Escherichia coli, as revealed by global metabolite pool ("metabolome") analysis, J. Bacteriol. 180 (1998) 5109–5116.
[11] S.G. Oliver, M.K. Winson, D.B. Kell, F. Baganz, Systematic functional analysis of the yeast genome, Trends Biotechnol. 16 (1998) 373–377.
[12] H. Tweeddale, L. Notley-McRobb, T. Ferenci, Assessing the effect of reactive oxygen species on Escherichia coli using a metabolome approach, Redox Rep. 4 (1999) 237–241.
[13] G.J. Patti, O. Yanes, G. Suizdak, Metabolomics: the apogee of the omic triology, Nat. Rev. Mol. Cell Biol. 14 (2013) 263–269.
[14] D.G. Robertson, U. Frevert, Metabolomics in drug discovery and development, Clin. Pharmacol. Ther. 94 (2013) 559–561.
[15] D.B. Kell, R. Goodacre, Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery, Drug Discov. Today 19 (2014) 171–182.
[16] J.D. Rabinowitz, J.G. Purdy, L. Vastag, T. Shenk, E. Koyuncu, Metabolomics in drug target discovery, Cold Spring Harb. Symp. Quant. Biol. 76 (2011) 235–246.
[17] R. Kaddurah-Daouk, K.R.R. Krishnan, Metabolomics: a global biochemical approach to the study of central nervous system diseases, Neuropsychopharmacol. 34 (2008) 173–186.
[18] M. Mamas, W.B. Dunn, L. Neyses, R. Goodacre, The role of metabolites and metabolomics in clinically applicable biomarkers of disease, Arch. Toxicol. 85 (2011) 5–17.
[19] M. Orešič, Metabolomics, a novel tool for studies of nutrition, metabolism, and lipid dysfunction, Nutr. Metab. Cardiovasc. Dis. 19 (2009) 816–824.
[20] H. Gibbons, A. O'Gorman, L. Brennan, Metabolomics as a tool in nutritional research, Curr. Opin. Lipidol. 26 (2015) (2015) 30–34.
[21] S.A. Goldansaz, A.C. Guo, T. Sajed, M.A. Steele, G.S. Plastow, D.S. Wishart, Livestock metabolomics and the livestock metabolome: a systematic review, PLoS One 12 (2017) 1–26.
[22] X. Qi, D. Zhang, Plant metabolomics and metabolic biology, J. Integr. Plant Biol. 56 (2014) 814–815.
[23] U.A. Meyer, U.M. Zanger, M. Schwab, Omics and drug response, Annu. Rev. Pharmacol. Toxicol. 53 (2013) 475–502.
[24] A. Julià, A. Alonso, S. Marsal, Metabolomics in rheumatic diseases, Int. J. Clin. Rheumatol. 9 (2014) 353–369.
[25] W.R. Wikoff, A.T. Anfora, J. Liu, P.G. Schultz, S.A. Lesley, E.C. Peters, G. Siuzdak, Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites, Proc. Natl. Acad. Sci. u.s.a. 106 (2009) 3698–3703.
[26] J.K. Nicholson, E. Holmes, J. Kinross, R. Burcetin, G. Gibson, W. Jia, S. Petterson, Host-gut microbiota metabolic interactions, Science 336 (2012) 1262–1267.
[27] K.N. Turi, L. Romick-Rosendale, K.K. Ryckman, T.V. Hartert, A review of metabolomics approaches and their application in identifying causal pathways of childhood asthma, J. Allergy Clin. Immunol. 141 (2018) 1191–1201.
[28] J. Zhou, Y. Yin, Strategies for large-scale targeted metabolomics quantification by liquid chromatography-mass spectrometry, Analyst 141 (2016) 6362–6373.
[29] D.Y. Lee, B.P. Bowen, T.R. Northen, Mass spectrometry-based metabolomics, analysis of metabolite-protein interactions, and imaging, Biotechniques 49 (2010) 557–565.
[30] W.B. Dunn, A. Erban, R.J.M. Weber, D.J. Creek, M. Brown, R. Breitling, T. Hankemeier, R. Goodacre, S. Neumann, J. Kopka, M.R. Viant, Mass appeal: metabolite identification in mass spectrometry-focused untargeted metabolomics, Metabolomics 9 (2013) 44–66.
[31] E.J. Want, I.D. Wilson, H. Gika, G. Theodoridis, R.S. Plumb, J. Shockor, E. Holmes, J.K. Nicholson, Global metabolic profiling procedures for urine using UPLC-MS, Nat. Protoc. 5 (2010) 1005–1018.
[32] N. Rifai, M.A. Gillette, S.A. Carr, Protein biomarker discovery and validation: the long and uncertain path to clinical utility, Nat. Biotechnol. 24 (2006) 971–983.
[33] L. Dang, D.W. White, S. Gross, B.D. Benneth, M.A. Bittinger, E.M. Driggers, V. R. Fantin, H.G. Jang, S. Jin, M.C. Keenan, K.M. Marks, R.M. Prins, P.S. Ward, K. E. Yen, L.M. Liau, J.D. Rabinowitz, L.C. Cantley, C.B. Thompson, M.G.V. Heiden, S.M. Su, Cancer-associated IDH1 mutations produce 2-hydroxyglutarate, Nature 462 (2009) 739.
[34] I.D. Wilson, J.K. Nicholson, Gut microbiome interactions with drug metabolism, efficacy and toxicity, Transl. Res. 179 (2017) 204–222.
[35] A. Saghatelian, S.A. Trauger, E.J. Want, E.G. Hawkins, G. Siuzdak, B.F. Cravatt, Assignment of endogenous substrates to enzymes by global metabolite profiling, Biochemistry 43 (2004) 14332–14339.
[36] A. Scalbert, L. Brennan, O. Fiehn, T. Hankemeier, B.S. Kristal, B.V. Ommen, E. Pujos-Guillot, E. Verheij, D. Wishart, S. Wopereis, Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research, Metabolomics 5 (2009) 435–458.
[37] J.P.A. Ioannidis, M.J. Khoury, Improving validation practices in 'omics' research, Science 334 (2011) 1230–1232.
[38] T. Kind, M. Scholz, O. Fiehn, How large is the metabolome? A critical analysis of data exchange practices in chemistry, PLoS One 4 (2009) e5440.
[39] S. Krug, G. Kastenmüller, F. Stückler, M.J. Rist, T. Skurk, M. Sailer, J. Raffler, W. Römisch-Margl, J. Adamski, C. Prehn, T. Frank, K. Engel, T. Hofmann, B. Luy, R. Zimmermann, F. Moritz, W. Szymczak, H. Hauner, K. Suhre, H. Daniel, The dynamic range of the human metabolome revealed by challenges, FASEB J. 26 (2012) 2607–2626.
[40] N. Psychogios, D.D. Hau, J. Peng, A.C. Guo, R. Mandal, S. Bouatra, I. Sinelnikov, R. Krishnamurthy, R. Eisner, B. Gautam, N. Young, J. Xia, C. Knox, E. Dong, P. Huang, Z. Hollander, T.L. Pedersen, S.R. Smith, F. Bamforth, R. Greiner, B. McManus, J.W. Newman, T. Goodfriend, D.S. Wishart, The human serum metabolome, PLoS One 6 (2011) e16957.
[41] G.A. Theodoridis, H.G. Gika, E.J. Want, I.D. Wilson, Liquid chromatography-mass spectrometry-based global metabolite profiling: a review, Anal. Chim. Acta 711 (2012) 7–16.
[42] L. Chen, F. Zhong, J. Zhu, Bridging targeted and untargeted mass spectrometry-based metabolomics via hybrid approaches, Metabolites 10 (2020) 1–19.
[43] W. Bin Xiao, Y. Jian, H. Li, Application of two-dimensional liquid chromatography in bioanalysis of drugs and toxicants, Chinese, J. Anal. Chem. 42 (2014) 1851–1858.
[44] J.W. Tomsho, A. Pal, D.G. Hall, S.J. Benkovic, Ring structure and aromatic substituent effects on the pKa of the benzoxaborole pharmacophore, ACS Med. Chem. Lett. 3 (2012) 48–52.
[45] A. Furey, M. Moriarty, V. Bane, B. Kinsella, M. Lehane, Ion suppression: a critical review on causes, evaluation, prevention and applications, Talanta 115 (2013) 104–122.
[46] T.M. Annesley, Ion suppression in mass spectrometry, Clin. Chem. 49 (2003) 1041–1044.
[47] A. Cappiello, G. Famiglini, P. Palma, H. Trufelli, Matrix effects in liquid chromatography-mass spectrometry, J. Liq. Chromatogr. Relat. Technol. 33 (2010) 1067–1081.
[48] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, Signal suppression/enhancement in high-performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1217 (2010) 3929–3937.
[49] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid, J. Am. Soc. Mass Spectrom. 14 (2003) 1290–1294.
[50] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[51] N.R. Srinivas, Dodging matrix effects in liquid chromatography-tandem mass spectrometric assays - compilation of key learnings and perspectives, Biomed. Chromatogr. 23 (2009) 451–454.

[52] T. Fuhrer, N. Zamboni, High-throughput discovery metabolomics, Curr. Opin. Biotechnol. 31 (2015) 73–78.
[53] S. Forcisi, F. Moritz, B. Kanawati, D. Tziotis, R. Lehmann, P. Schmitt-Kopplin, Liquid chromatography-mass spectrometry in metabolomics research: mass analyzers in ultra-high-pressure liquid chromatography coupling, J. Chromatogr. A 1292 (2013) 51–65.
[54] D.D. Marshall, R. Powers, Beyond the paradigm: combining mass spectrometry and nuclear magnetic resonance for metabolomics, Progr. Nuclear Mag, Resonance 100 (2017) 1–16.
[55] A.A. Crook, R. Powers, Quantitative NMR-based biomedical metabolomics: current status and applications, Molecules 25 (2020) 5128.
[56] D.S. Wishart, L.L. Cheng, V. Copie, A.S. Edison, H.R. Eghbalnia, J.C. Hoch, G. J. Gouveia, W. Pathmasiri, R. Powers, T.B. Schock, L.W. Sumner, M. Uchimiya, NMR and metabolomics - a roadmap for the future, Metabolites 12 (2022) 678.
[57] O. Beckonert, H.C. Keun, T.M.D. Ebbels, J. Bundy, E. Holmes, J.C. Lindon, J. K. Nicholson, Metabolic profiling, metabolic and metabonomic procedurs for NMR spectroscopy of urine, plasma, serum and tissue extracts, Nature Protocols 2 (2007) 2692–2703.
[58] G.A.N. Gowda, D. Raftery, Quantitating metabolites in protein precipitated serum using NMR spectroscopy, Anal. Chem. 86 (2014) 5433–5440.
[59] J.K. Nicholson, J.C. Lindon, E. Holmes, 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data, Xenobiotica 29 (1999) 1181–1189.
[60] S. Alseekh, A. Aharoni, Y. Brotman, K. Contrepois, J. D'Auria, J. Ewald, J. C. Ewald, P.D. Fraser, P. Giavalisco, R.D. Hall, M. Heinemann, H. Link, J. Luo, S. Neumann, J. Nielsen, L.P. de Souza, K. Saito, U. Sauer, F.C. Schroeder, S. Schuster, G. Siuzdak, A. Skirycz, L.W. Sumner, M.P. Snyder, H. Tang, T. Tohge, Y. Wang, W. Wen, S. Wu, G. Xu, N. Zamboni, A.R. Fernie, Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices, Nat. Methods 18 (2021) 747–756.
[61] A. Vignoli, V. Ghini, G. Meoni, C. Licari, P.G. Takis, L. Tenori, P. Turano, C. Luchinat, High-throughput metabolomics by 1D NMR, Agnew. Chem. Int. Ed. 58 (2019) 968–994.
[62] J.P. Shockcor, S.E. Unger, I.D. Wilson, P.J.D. Foxall, J.K. Nicholson, J.C. Lindon, Combined HPLC, NMR spectroscopy, and ion-trap mass spectrometry with application to the detection and characterization of xenobiotic and endogeneous metabolites in human urine, Anal. Chem. 68 (1996) 4431–4435.
[63] J.P. Shockcor, S.E. Unger, P. Savina, J.K. Nicholson, J.C. Lindon, Application of directly coupled LC-NMR-MS to the structural elucidation of metabolites of the HIV-1 reverse-transcriptase inhibitor BW935U83, J. Chromatogr. B 748 (2000) 269–279.
[64] E.J. Want, A. Nordström, H. Morita, G. Siuzdak, From exogenous to endogenous: the inevitable imprint of mass spectrometry in metabolomics, J. Proteome Res. 6 (2007) 459–468.
[65] J. Gullberg, P. Jonsson, A. Nordström, M. Sjöström, T. Moritz, Design of experiments: an efficient strategy to identify factors influencing extraction and derivatization of Arabidopsis thaliana samples in metabolomic studies with gas chromatography/mass spectrometry, Anal. Biochem. 331 (2004) 283–295.
[66] W. Lu, B.D. Bennett, J.D. Rabinowitz, Analytical strategies for LC-MS-based targeted metabolomics, J. Chromatogr. B 871 (2008) 236–242.
[67] J. Lisec, N. Schauer, J. Kopka, L. Willmitzer, A.R. Fernie, Gas chromatography-mass spectrometry-based metabolite profiling in plants, Nat. Protoc. 1 (2006) 387–396.
[68] M.R.N. Monton, T. Soga, Metabolome analysis by capillary electrophoresis-mass spectrometry, J. Chromatogr. 1168 (2007) 237–246.
[69] S.U. Bajad, W. Lu, E.H. Kimball, J. Yuan, C. Peterson, J.D. Rabinowitz, Separation and quantitation of water-soluble cellular metabolites by hydrophilic interaction chromatography-tandem mass spectrometry, J. Chromatogr. A 1125 (2006) 76–88.
[70] D.B. Kell, Metabolomics and systems biology: making sense of soup, Curr. Opin. Microbiol. 7 (2004) 296–307.
[71] R.A. Iles, R.A. Chalmers, S.P. Burns, High-resolution 1H-NMR spectroscopy of blood plasma for metabolic studies, Clin. Chem. 41 (1995) 1054–1056.
[72] E.L. Rodriguez, S. Poddar, S. Iftekhar, K. Suh, A.G. Woolfork, S. Ovbude, A. Pekarek, M. Walters, S. Lott, D.S. Hage, Affinity chromatography: a review of trends and developments over the past 50 years, J. Chromatogr. B 1157 (2020) 122332.
[73] C. Zheng, S. Zhang, S. Ragg, D. Raftery, O. Vitek, Identification and quantification of metabolites in (1)H NMR spectra by Bayesian model selection, Bioinformatics 27 (2011) 1637–1644.
[74] J.-L. Ren, A.-H. Zhang, L. Kong, X.-J. Wang, Advances in mass spectrometry-based metabolomics for invesigation of metabolites, RSC Adv. 8 (2018) 22335–22350.
[75] R. Gonzalez-Dominguez, A. Sayago, A. Fernandez-Recamales, Direct infusion mass spectrometry for metabolic phenotyping of diseases, Future Sci. 9 (2016) 131–148.
[76] L. Lin, Q. Yu, X. Yan, W. Hang, J. Zheng, J. Xing, B. Huang, Direct infusion mass spectrometry or liquid chromatography mass spectrometry for human metabolomics? A serum metabonomic study of kidney cancer, Analyst 135 (2010) 2970–2978.
[77] I. Aretz, D. Meierhofer, Advantages and pitfalls of mass spectrometry based metabolome profiling in systems biology, Int. J. Mol. Sci. 17 (2016) 632.
[78] M.E.C. Queiroz, I.D. Souza, Restricted access media, in: C. Poole (Ed.), Handbooks in Separation Science, Solid-Phase Extraction, Elsevier Inc., 2020, pp. 129–149.
[79] T. Cajka, O. Fiehn, Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics, Anal. Chem. 88 (2016) 524–545.
[80] T. Cajka, O. Fiehn, Increasing lipidomic coverage by selecting optimal mobile-phase modifiers in LC-MS of blood plasma, Metabolomics 12 (2016) 1–11.
[81] L. Whiley, J. Godzien, F.J. Ruperez, C. Legido-Quigley, C. Barbas, In-vial dual extraction for direct LC-MS analysis of plasma for comprehensive and highly reproducible metabolic fingerprinting, Anal. Chem. 84 (2012) 5992–5999.
[82] J. Godzien, M. Ciborowski, L. Whiley, C. Legido-Quigley, F.J. Ruperez, C. Barbas, In-vial dual extraction liquid chromatography coupled to mass spectrometry applied to streptozotocin-treated diabetic rats. Tips and pitfalls of the method, J. Chromatogr. A 1304 (2013) 52–60.
[83] C. Andresen, T. Boch, H.M. Gegner, N. Mechtel, A. Narr, E. Birgin, E. Rasbach, N. Rahbari, A. Trumpp, G. Poschet, D. Hubschmann, Comparison of extration methods for intracellular metabolomics of human tissues, Front. Mol. Biosci. 9 (2022) 932261.
[84] M.Y. Mushtaq, Y.H. Choi, R. Verpoorte, E.G. Wilson, Extraction for metabolomics: access to the metabolome, Phytochem, Analysis 25 (2014) 291–306.
[85] M. Breier, S. Wahl, C. Prehn, M. Fugmann, U. Ferrari, M. Weise, F. Banning, J. Seissler, H. Grallert, J. Adamski, A. Lechner, Targeted metabolomics identifies reliable and stable metabolites in human serum and plasma samples, PLOS One 9 (2014) e89728.
[86] M.V. Fomenko, L.V. Yanshole, Y.P. Tsentalovich, Stability of metabolomic content during sample pretreatment: blood and brain tissues, Metabolites 12 (2022) 811.
[87] A. Gil, D. Siegel, H. Permentier, D.-J. Reijngoud, F. Dekker, R. Bischoff, Stability of energy metabolites - an often overlooked issue in metabolomics studies: a review, Electrophoresis 36 (2015) 2156–2169.
[88] V.L. Stevens, E. Hoover, Y. Wang, K.A. Zanetti, Pre-analytical factors that affect stability in human urine, plasma, and serum: a review, Metabolites 9 (2019) 156.
[89] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, Optimisation and routine use of generic ultra-high flow-rate liquid chromatography with mass spectrometric detection for the direct on-line analysis of pharmaceuticals in plasma, J. Chromatogr. A 828 (1998) 199–207.
[90] D. Vuckovic, Current trends and challenges in sample preparation for global metabolomics using liquid chromatography-mass spectrometry, Anal. Bioanal. Chem. 403 (2012) 1523–1548.
[91] J.K. Nicholson, K.P.R. Gartland, 1H NMR studies on protein binding of histidine, tyrosine and phenylalanine in blood plasma, NMR Biomed. 2 (1989) 77–82.
[92] J.D. Bell, J.C. Brown, G. Kubal, P.J. Sadler, NMR-invisible lactate in blood plasma, FEBS Lett. 235 (1988) 81–86.
[93] M. Gil, S. Samino, R. Barrilero, X. Correig, Lipid profiling using ^{1}H NMR spectroscopy, Methods Mol. Biol. 2037 (2019) 35–47.
[94] P. Le Pogam, A. Pillot, F. Lohezic-Le Devehat, A.-C. Le Lamer, B. Legouin, A. Gadea, A. Sauvager, D. Ertz, J. Boustie, Mass spectrometry as a versatile ancillary technique for the rapid in situ identification of lichen metabolites directly from TLC plates, Lichenol. 49 (2017) 507–520.
[95] I.D. Wilson, C.F. Poole, Planar chromatography – current practice and future prospects, J. Chromatogr. B 1214 (2022) 123553.
[96] D.S. Hage, J.D. Carr, Analytical Chemistry and Quantitative Analysis, Prentice Hall, New York, 2011.
[97] D.S. Hage, Chromatography, in: N. Rifai, A.R. Horvath, C.T. Wittwer (Eds.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th Ed., Elsevier, Amsterdam, 2018, pp. 266–294.
[98] C.F. Poole, S.K. Poole, Chromatography Today, Elsevier, New York, 1991.
[99] S.P. Dixon, I.D. Pitfield, D. Perrett, Comprehensive multi-dimensional liquid chromatographic separation in biomedical and pharmaceutical analysis, Biomed. Chromatogr. 20 (2006) 508–529.
[100] A. Kloppel, W. Grasse, F. Brummer, G.E. Morlock, HTPLC coupled with bioluminescence and mass spectrometry for bioactivity-based analysis of secondary metabolites in marine sponges, J. Planar Chromatogr. 21 (2008) 431–436.
[101] M. Li, Z. Zhou, H. Nie, Y. Bai, H. Liu, Recent advances of chromatography and mass spectrometry in lipidomics, Anal. Bioanal. Chem. 299 (2011) 243–249.
[102] B. Fuchs, J. Schiller, R. Suss, M. Zscharnack, A. Bader, P. Muller, M. Schuenberg, M. Becker, D. Suckau, Analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS, Anal. Bioanal. Chem. 392 (2008) 849–860.
[103] N. Gogo-Inoue, T. Hayaska, T. Taki, T.V. Gonzalez, M. Setou, A new lipidomics approach by thin-layer chromatography-blot-matrix-assisted laser desorption/ ionization imaging mass spectrometry for analyzing detailed patterns of phospholipid molecular species, J. Chromatogr. A 1216 (2009) 7096–7101.
[104] G. Stubiger, E. Pittenauer, O. Belgacem, P. Rehulka, K. Widhalm, G. Allmaier, Analysis of human plasma lipids and soybean lecithin by means of high-performance thin-layer chromatography and matrix-assisted laser desorption/ ionization mass spectrometry, Rapid. Commun. Mass Spectrom. 23 (2009) 2711–2723.
[105] G. Paglia, D.R. Ifa, C.P. Wu, G. Corso, R.G. Cook, Thin-layer chromatography and mass spectrometry coupled using desorption electrospray ionization, Anal. Chem. 82 (2010) 1744–1750.
[106] A. Maruška, O. Kornyšova, Application of monolithic (continuous bed) chromatographic columns in phytochemical analysis, J. Chromatogr. A 1112 (2006) 319–330.
[107] M. Rodriguez-Aller, R. Gurny, J.L. Veuthey, D. Guillarme, Coupling ultra-high-pressure liquid chromatography with mass spectrometry: constraints and possible applications, J. Chromatogr. A 1292 (2016) 2–18.

[108] D. Guillarme, D.T.T. Nguyen, S. Rudaz, J.L. Veuthey, Recent developments in liquid chromatography-impact on qualitative and quantitative performance, J. Chromatogr. A 1149 (2007) 20–29.
[109] I.D. Wilson, J.K. Nicholson, J. Castro-Perez, J.H. Granger, K.A. Johnson, B. W. Smith, R.S. Plumb, High resolution 'ultra performance' liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies, J. Proteome Res. 4 (2005) 591–598.
[110] D.T.T. Nguyen, D. Guillarme, S. Rudaz, J.L. Veuthey, Fast analysis in liquid chromatography using small particle size and high pressure, J. Sep. Sci. 29 (2006) 1836–1848.
[111] K. Yu, D. Little, R. Plumb, B. Smith, High-throughput quantification for a drug mixture in rat plasma - a comparison of ultra performance liquid chromatography/tandem mass spectrometry with high-performance liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 20 (2006) 544–552.
[112] J.J. Pitt, Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry, Clin Biochem Rev. 30 (2009) 19–34.
[113] T.M. Annesley, Methanol-associated matrix effects in electrospray ionization tandem mass spectrometry, Clin. Chem. 53 (2007) 1827–1834.
[114] J. Martens-Lobenhoffer, S.M. Bode-Böger, Fast and efficient determination of arginine, symmetric dimethylarginine, and asymmetric dimethylarginine in biological fluids by hydrophilic-interaction liquid chromatography-electrospray tandem mass spectrometry, Clin. Chem. 52 (2006) 488–493.
[115] R. Oertel, V. Neumeister, W. Kirch, Hydrophilic interaction chromatography combined with tandem mass spectrometry to determine six aminoglycosides in serum, J. Chromatogr. A 1058 (2004) 197–201.
[116] P.A. Aronov, L.M. Hall, K. Dettmer, C.B. Stephensen, B.D. Hammock, Metabolic profiling of major vitamin D metabolites using Diels-Alder derivatization and ultra-performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 391 (2008) 1917–1930.
[117] H. Licea-Perez, S. Wang, M.E. Szapacs, E. Yang, Development of a highly sensitive and selective UPLC/MS/MS method for the simultaneous determination of testosterone and 5α-dihydrotestosterone in human serum to support testosterone replacement therapy for hypogonadism, Steroids 73 (2008) 601–610.
[118] R. Ventura, M. Roig, N. Monfort, P. Sáez, R. Bergés, J. Seguraab, High-throughput and sensitive screening by ultra-performance liquid chromatography-tandem mass spectrometry of diuretics and other doping agents, Eur. J. Mass Spectrom. 14 (2008) 191–200.
[119] P. Yin, D. Wan, C. Zhao, J. Chen, X. Zhao, W. Wang, X. Lu, S. Yang, J. Gu, G. Xu, A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry, Mol. Biosyst. 5 (2009) 868–876.
[120] Y. Wang, X. Lu, G. Xu, Simultaneous separation of hydrophilic and hydrophobic compounds by using an online HILIC-RPLC system with two detectors, J. Sep. Sci. 31 (2008) 1564–1572.
[121] J. Chen, W. Wang, S. Lv, P. Yin, X. Zhan, X. Lu, F. Zhang, G. Xu, Metabonomics study of liver cancer based on ultra-performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations, Anal. Chim. Acta 650 (2009) 3–9.
[122] L. Zhong, F. Cheng, X. Lu, Y. Duan, X. Wang, Untargeted saliva metabonomics study of breast cancer based on ultra-performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations, Talanta 158 (2016) 351–360.
[123] A. Lafaye, J. Labarre, J.C. Tabet, E. Ezan, C. Junot, Liquid chromatography-mass spectrometry and 15N metabolic labeling for quantitative metabolic profiling, Anal. Chem. 77 (2005) 2026–2033.
[124] S. Fekete, J. Schappler, J.L. Veuthey, D. Guillarme, Current and future trends in UHPLC, Trends Anal. Chem. 63 (2014) 2–13.
[125] H. Wang, R. Huang, H. Li, L. Jiao, S. Liu, W. Wu, Serum metabolomic analysis of the anti-diabetic effect of Ginseng berry in type II diabetic rats based on ultra-high-performance liquid chromatography-high resolution mass spectrometry, J. Pharm. Biomed. Anal. 196 (2021) 1–10.
[126] M.I. Churchwell, N.C. Twaddle, L.R. Meeker, D.R. Doerge, Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS, J. Chromatogr. B 825 (2005) 134–143.
[127] A. Nordström, G. O'Maille, C. Qin, G. Siuzdak, Nonlinear data alignment for UPLC-MS and HPLC-MS based metabolomics: quantitative analysis of endogenous and exogenous metabolites in human serum, Anal. Chem. 78 (2006) 3289–3295.
[128] J.J. Kirkland, S.A. Schuster, W.L. Johnson, B.E. Boyes, Fused-core particle technology in high-performance liquid chromatography: an overview, J. Pharm. Anal. 3 (2013) 303–312.
[129] M. Rigobello-Masini, J.C.P. Penteado, J.C. Masini, Monolithic columns in plant proteomics and metabolomics, Anal. Bioanal. Chem. 405 (2013) 2107–2122.
[130] D.P. Kloos, H. Lingeman, W.M.A. Niessen, A.M. Deelder, M. Giera, O. A. Mayboroda, Evaluation of different column chemistries for fast urinary metabolic profiling, J. Chromatogr. B 927 (2013) 90–96.
[131] V. V. Tolstikov, A. Lommen, K. Nakanishi, N. Tanaka, O. Fiehn, Monolithic silica-based capillary reversed-phase liquid chromatography/electrospray mass spectrometry for plant metabolomics.
[132] I.D. Wilson, High-performance liquid chromatography-mass spectrometry (HPLC-MS)-based drug metabolite profiling, Methods Mol. Biol. 708 (2011) 173–190.
[133] A. Triolo, M. Altamura, T. Dimoulas, A. Guidi, A. Lecci, M. Tramontana, In vivo metabolite detection and identification in drug discovery via LC-MS/MS with data-dependent scanning and postacquisition data mining, J. Mass Spectrom. 40 (2005) 1572–1582.
[134] R.S. Plumb, J.H. Granger, C.L. Stumpf, K.A. Johnson, B.W. Smith, S. Gaulitz, I. D. Wilson, J. Castro-Perez, A rapid screening approach to metabonomics using UPLC and oa-TOF mass spectrometry: application to age, gender and diurnal variation in normal/Zucker obese rats and black, white and nude mice, Analyst 130 (2005) 844–849.
[135] N.J. Clarke, D. Rindgen, W.A. Korfmacher, K.A. Cox, Systematic LC/MS metabolite identification in drug discovery, Anal. Chem. 73 (2001) 430A–A439.
[136] J.M. Castro-Perez, Current and future trends in the application of HPLC-MS to metabolite-identification studies, Drug Discov. Today 12 (2007) 249–256.
[137] N.P. Long, T.D. Nghi, Y.P. Kang, N.H. Anh, H.M. Kim, S.K. Park, S.W. Kwon, Toward a standardized strategy of clinical metabolomics for the advancement of precision medicine, Metabolites 10 (2020) 51.
[138] B. Yu, K.A. Zanetti, M. Temprosa, D. Albanes, N. Appel, C.B. Barrera, Y. Ben-Shlomo, E. Boerwinkle, J.P. Casas, C. Clish, C. Dale, A. Dehghan, A. Derkach, A. H. Eliassen, P. Elliott, E. Fahy, C. Gieger, M.J. Gunter, S. Harada, T. Harris, D. R. Herr, D. Herrington, J.N. Hirschhorn, E. Hoover, A.W. Hsing, M. Johansson, R. S. Kelly, C.M. Khoo, M. Kivimäki, B.S. Kristal, C. Langenberg, J. Lasky-Su, D. A. Lawlor, L.A. Lotta, M. Mangino, L. Le Marchand, E. Mathé, C.E. Matthews, C. Menni, L.A. Mucci, R. Murphy, M. Oresic, E. Orwoll, J. Ose, A.C. Pereira, M. C. Playdon, L. Poston, J. Price, Q. Qi, K. Rexrode, A. Risch, J. Sampson, W. Jie Seow, H.D. Sesso, S.H. Shah, X.-O. Shu, G.C.S. Smith, U. Sovio, V.L. Stevens, R. Stolzenberg-Solomon, T. Takebayashi, T. Tillin, R. Travis, I. Tzoulaki, C. M. Ulrich, R.S. Vasan, M. Verma, Y. Wang, N.J. Wareham, A. Wong, N. Younes, H. Zhao, W. Zheng, S.C. Moore, The Consortium of Metabolomics Studies (COMET): metabolomics in 47 prospective cohort studies, Am. J. Epidemiol. 188 (2019) 991–1012.
[139] E. Stancliffe, M. Schwaiger-Haber, M. Sindelar, M.J. Murphy, M. Soerensen, G. J. Patti, An untargeted metabolomics workflow that scales to thousands of samples for population-based studies, Anal. Chem. 94 (2022) 17370–17378.
[140] D.S. Wishart, A. Guo, E. Oler, F. Wang, A. Anjum, H. Peters, R. Dizon, Z. Sayeeda, S. Tian, B.L. Lee, M. Berjanskii, R. Mah, M. Yamamoto, J. Jovel, C. Torres-Calzada, M. Hiebert-Giesbrecht, V.W. Lui, D. Varshavi, D. Varshavi, D. Allen, D. Arndt, N. Khetarpal, A. Sivakumaran, K. Harford, S. Sanford, K. Yee, X. Cao, Z. Budinski, J. Liigand, L. Zhang, J. Zheng, R. Mandal, N. Karu, M. Dambrova, H. B. Schiöth, R. Greiner, V. Gautam, HMDB 5.0: the human metabolome database for 2022, Nucl. Acids Res. 50 (2022) D622–D631.
[141] H.J. Issaq, Q.N. Van, T.J. Waybright, G.M. Muschik, T.D. Veenstra, Analytical and statistical approaches to metabolomics research, J. Sep. Sci. 32 (2009) 2183–2199.
[142] J.N. Fairchild, K. Horvath, J.R. Gooding, S.R. Campagna, G. Guiochon, Two-dimensional liquid chromatography/mass spectrometry/mass spectrometry separation of water-soluble metabolites, J. Chromatogr. A 1217 (2010) 8161–8166.
[143] D.R. Stoll, X. Li, X. Wang, P.W. Carr, S.E.G. Porter, S.C. Rutan, Fast, comprehensive two-dimensional liquid chromatography, J. Chromatogr. A 3–43 (2007).
[144] B.W.J. Pirok, D.R. Stoll, P.J. Schoenmakers, Recent developments in two-dimensional liquid chromatography: fundamental improvements for practical applications, Anal. Chem. 91 (2019) 240–263.
[145] D. Stoll, J. Danforth, K. Zhang, A. Beck, Characterization of therapeutic antibodies and related products by two-dimensional liquid chromatography coupled with UV absorbance and mass spectrometric detection, J. Chromatogr. B 1032 (2016) 51–60.
[146] K. Sandra, P. Sandra, Lipidomics from an analytical perspective, Curr. Opin. Chem. Biol. 17 (2013) 847–853.
[147] M.A.I. Prodhan, B. Shi, M. Song, L. He, F. Yuan, X. Yin, B. Bohman, C.J. McClain, X. Zhang, Integrating comprehensive two-dimensional gas chromatography-mass spectrometry and parallel two-dimensional liquid chromatography-mass spectrometry for untargeted metabolomics, Analyst 144 (2019) 4331–4341.
[148] M.M. Koek, F.M. van der Kloet, R. Kleemann, T. Kooistra, E.R. Verheij, T. Hankemeier, Semi-automated non-target processing in GC × GC-MS metabolomics analysis: Applicability for biomedical studies, Metabolomics 7 (2011) 1–14.
[149] A.N.T. Phan, L.M. Blank, GC-MS-based metabolomics for the smut fungus Ustilago maydis: a comprehensive method optimization to quantify intracellular metabolites, Front. Mol. Biosci. 7 (2020) 1–10.
[150] R. Tomas, K. Kleparnik, F. Foret, Multidimensional liquid phase separations for mass spectrometry, J. Sep. Sci. 31 (2008) 1964–1979.
[151] M. Navarro-Reig, J. Jaumot, A. Baglai, G. Vivó-Truyols, P.J. Schoenmakers, R. Tauler, Untargeted comprehensive two-dimensional liquid chromatography coupled with high-resolution mass spectrometry analysis of rice metabolome using multivariate curve resolution, Anal. Chem. 89 (2017) 7675–7683.
[152] K. Contrepois, L. Jiang, M. Snyder, Optimized analytical procedures for the untargeted metabolomic profiling of human urine and plasma by combining hydrophilic interaction (HILIC) and reverse-phase liquid chromatography (RPLC)-mass spectrometry, Mol. Cell. Proteomics 14 (2015) 1684–1695.
[153] L. Zhong, F. Cheng, X. Lu, Y. Duan, X. Wang, Untargeted saliva metabonomics study of breast cancer based on ultra performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations, Talanta 158 (2016) 351–360.
[154] W. Liu, Q. Song, Y. Cao, Y. Zhao, H. Huo, Y. Wang, Y. Song, J. Li, P. Tu, Advanced liquid chromatography-mass spectrometry enables merging widely targeted metabolomics and proteomics, Anal. Chim. Acta 1069 (2019) 89–97.
[155] S. Boudah, M.-F. Olivier, S. Aros-Calt, L. Oliveira, F. Fenaille, J.-C. Tabet, C. Junot, Annotation of the human serum metabolome by coupling three liquid

chromatography methods to high-resolution mass spectrometry, J. Chromatogr. B 966 (2014) 34–47.
[156] D. Hemmler, S.S. Heinzmann, K. Wöhr, P. Schmitt-Kopplin, M. Witting, Tandem HILIC-RP liquid chromatography for increased polarity coverage in food analysis, Electrophoresis 39 (2018) 1645–1653.
[157] K. Spagou, I.D. Wilson, P. Masson, G. Theodoridis, N. Raikos, M. Coen, E. Holmes, J.C. Lindon, R.S. Plumb, J.K. Nicholson, E.J. Want, HILIC-UPLC-MS for exploratory urinary metabolic profiling in toxicological studies, Anal. Chem. 83 (2011) 382–390.
[158] P.A. Vorkas, G. Isaac, M.A. Anwar, A.H. Davies, E.J. Want, J.K. Nicholson, E. Holmes, Untargeted UPLC-MS profiling pipeline to expand tissues metabolome coverage: application to cardiovascular disease, Anal. Chem. 87 (2015) 4184–4193.
[159] M. Holčapek, M. Ovčačíková, M. Lísa, E. Cífková, T. Hájek, Continuous comprehensive two-dimensional liquid chromatography-electrospray ionization mass spectrometry of complex lipidomic samples, Anal. Bioanal. Chem. 407 (2015) 5033–5043.
[160] M.J. Berna, B.L. Ackermann, A.T. Murphy, High-throughput chromatographic approaches to liquid chromatographic/ tandem mass spectrometric bioanalysis to support drug discovery and development, Anal. Chim. Acta 509 (2004) 1–9.
[161] M. Zell, C. Husser, G. Hopfgartner, Column-switching high-performance liquid chromatography combined with ion spray tandem mass spectrometry for the simultaneous determination of the platelet inhibitor Ro 44–3888 and its pro-drug and precursor metabolite in plasma, J. Mass Spectrom. 32 (1997) 23–32.
[162] J.I. Yamaguchi, K. Hachiuma, Y. Kimura, N. Ogawa, S. Higuchi, Highly sensitive determination of TS-962 (HL-004) a novel acyl-CoA: cholesterol acyltransferase inhibitor, in rat and rabbit plasma by liquid chromatography and atmospheric pressure chemical ionization tandem mass spectrometry combined with a column-switch, J. Chromatogr. B 750 (2001) 99–108.
[163] Y.Q. Xia, C.E.C.A. Hop, D.Q. Liu, S.H. Vincent, S.H.L. Chiu, Parallel extraction columns and parallel analytical columns coupled with liquid chromatography/ tandem mass spectrometry for online simultaneous quantification of a drug candidate and its six metabolites in dog plasma, Rapid Commun. Mass Spectrom. 15 (2001) 2135–2144.
[164] C. Fernández-Ramos, D. Šatínský, P. Solich, New method for the determination of carbamate and pyrethroid insecticides in water samples using on-line SPE fused core column chromatography, Talanta 129 (2014) 579–585.
[165] I. Brabcová, M. Hlaváčková, D. Šatínský, P. Solich, A rapid HPLC column switching method for sample preparation and determination of β-carotene in food supplements, Food Chem. 141 (2013) 1433–1437.
[166] X. Zhang, H. Hou, H. Chen, Y. Liu, A. Wang, Q. Hu, A column-switching LC-MS/MS method for simultaneous quantification of biomarkers for 1,3-butadiene exposure and oxidative damage in human urine, J. Chromatogr. B 1002 (2015) 123–129.
[167] E.H. Ji, J. Lee, S. Hu, Ion chromatography with mass spectrometry for metabolomic analysis, in: S. Hu (Ed.), Cancer Metabolomics, Springer Nature Switzerland AG, Basel, 2021, pp. 149–159.
[168] P. Žuvela, M. Skoczylas, J.J. Liu, T. Baczek, R. Kaliszan, M.W. Wong, B. Buszewski, Column characterization and selection systems in reversed-phase high-performance liquid chromatography, Chem. Rev. 119 (2019) 3674–3729.
[169] R.E. Majors, P.W. Carr, Glossary of liquid-phase separation terms, LC-GC 19 (2001) 124–162.
[170] C. Boone, J. Adamec, Top-down proteomics, in: P. Ciborowski, J. Silberring (Eds.), Proteomic Profiling and Analytical Chemistry, 2nd Ed., Elsevier, Amsterdam, 2016, pp. 175–191.
[171] J. Sjovall, M. Axelson, Newer approaches to the isolation, identification, and quantitation of steroids in biological matrices, Vitamins and Hormones 39 (1982) 31–144.
[172] S. Liu, J. Sjovall, W.J. Griffiths, Neurostroids in rat brain: extraction, isolation, and analysis by nanoscale liquid chromatography-electrospray mass spectrometry, Anal. Chem. 75 (2003) 5835–5846.
[173] P. Liere, A. Pianos, B. Eychenne, A. Cambourg, K. Bodin, W. Griffiths, M. Schumacher, E.-E. Baulieu, J. Sjovall, Analysis of pregnenolone an dehydroepiandrosterone in rodent brain: cholesterol oxidation is the key, J. Lipid Res. 50 (2009) 2430–2444.
[174] H. Yoshida, T. Mizukoshi, K. Hirayama, H. Miyano, On-line desalting-mass spectrometry system for the structural determination of hydrophilic metabolites, using a column switching technique and a volatile ion-pairing reagent, J. Chromatogr. A 1119 (2006) 315–321.
[175] T. Piper, W. Schänzer, M. Thevis, Revisiting the metabolism of 19-nortestosterone using isotope ratio and high resolution/high accuracy mass spectrometry, J. Steroid Biochem. Mol. Biol. 162 (2016) 80–91.
[176] S.B. Lee, Y.K. Rhee, F.J. Gu, D.W. Kim, G.J. Jang, S.H. Song, J.I. Lee, H.D. Hong, C.W. Cho, H.J. Kim, Mass-based metabolomic analysis of lactobacillus sakei and its growth media at different growth phases, J. Microbiol. Biotechnol. 27 (2017) 925–932.
[177] S. Wang, L. Zhou, Z. Wang, X. Shi, G. Xu, Simultaneous metabolomics and lipidomics analysis based on novel heart-cutting two-dimensional liquid chromatography-mass spectrometry, Anal. Chim. Acta 966 (2017) 34–40.
[178] D.J. Floros, K. Xu, F. Berthiller, H. Schwartz-Zimmerman, Comparison of chromatographic conditions for the targeted tandem mass spectrometric determination of 354 mammalian metabolites, J. Chromatogr. A 1697 (2023) 463985.
[179] A.J. Alpert, Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids, and other polar compounds, J. Chromatogr. A 499 (1990) 177–196.
[180] M.R. Gama, R.G. da Costa Silva, C.H. Collins, C.B.G. Bottoli, Hydrophilic interaction chromatography, Trends Anal. Chem. 37 (2012) 48–60.
[181] B.A. Olsen, Hydrophilic interaction chromatography using amino and silica columns for the determination of polar pharmaceuticals and impurities, J. Chromatogr. A 913 (2001) 113–122.
[182] B. Buszewski, S. Noga, Hydrophilic interaction liquid chromatography (HILIC)-a powerful separation technique, Anal. Bioanal. Chem. 402 (2012) 231–247.
[183] V.V. Tolstikov, O. Fiehn, Analysis of highly polar compounds of plant origin: combination of hydrophilic interaction chromatography and electrospray ion trap mass spectreometry, Anal. Biochem. 301 (2002) 298–307.
[184] F. Fei, D.M.E. Bowdish, B.E. McCarry, Comprehensive and simultaneous coverage of lipid and polar metabolites for endogenous cellular metabolomics using HILIC-TOF-MS, Anal. Bioanal. Chem. 406 (2014) 3723–3733.
[185] K. Spagou, H. Tsoukali, N. Raikos, H. Gika, I.D. Wilson, G. Theodoridis, Hydrophilic interaction chromatography coupled to MS for metabonomic/ metabolomic studies, J. Sep. Sci. 33 (2010) 716–727.
[186] H. Bi, K.W. Krausz, S.K. Manna, F. Li, C.H. Johnson, F.J. Gonzalez, Optimization of harvesting, extraction, and analytical protocols for UPLC-ESIMS-based metabolomic analysis of adherent mammalian cancer cells, Anal. Bioanal. Chem. 405 (2013) 5279–5789.
[187] W. Zou, J. She, V. Tolstikov, A comprehensive workflow of mass spectrometry-based untargeted metabolomics in cancer metabolic biomarker discovery using human plasma and urine, Metabolites 3 (2013) 787–819.
[188] M. Urpi-Sarda, M. Monagas, N. Khan, R. Llorach, R.M. Lamuela-Raventos, O. Jauregui, R. Estruch, M. Izquierdo-Pulido, C. Andres-Lacueva, Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 7258–7267.
[189] I. Kohler, R.J.E. Derks, M. Giera, The rise of hydrophilic interaction chromatography in untargeted clinical metabolomics, LC-GC Eur. 29 (2016) 60–75.
[190] D.Q. Tang, L. Zou, X.X. Yin, C.N. Ong, HILIC-MS for metabolomics: an attractive and complementary approach to RPLC-MS, Mass Spectrom. Rev. 35 (2016) 574–600.
[191] G. Greco, T. Letzel, Main interactions and influences of the chromatographic parameters in HILIC separations, J. Chromatogr. Sci. 51 (2013) 684–693.
[192] C.B. Newgard, J. An, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, L.F. Lien, A. M. Haqq, S.H. Shah, M. Arlotto, C.A. Slentz, J. Rochon, D. Gallup, O. Ilkaeva, B. R. Wenner, W.E. Yancy, H. Eisenson, G. Musante, R. Surwit, D.S. Millington, M. D. Butter, L.P. Svetke, A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance, Cell Metab. 9 (2009) 311–326.
[193] C.S. McCoin, T.A. Knotts, S.H. Adams, Acylcarnitines-old actors auditioning for new roles in metabolic physiology, Nat. Rev. Endocrinol. 11 (2015) 617–625.
[194] T. Teav, H. Gallart-Ayala, V. Van Der Velpen, F. Mehl, H. Henry, J. Ivanisevic, Merged targeted quantification and untargeted profiling for comprehensive assessment of acylcarnitine and amino acid metabolism, Anal. Chem. 91 (2019) 11757–11769.
[195] P. Yin, G. Xu, Current state-of-the-art of nontargeted metabolomics based on liquid chromatography-mass spectrometry with special emphasis on clinical applications, J. Chromatogr. A 1374 (2014) 1–13.
[196] M. Kaczmarek, N. Zhang, L. Buzhansky, S. Gilead, E. Gazit, Optimization strategies for mass spectrometry-based untargeted metabolomics analysis of small polar molecules in human plasma, Metabolites 13 (2023) 923.
[197] M.R. Wenk, The emerging field of lipidomics, Nature Rev. Drug Discov. 4 (2005) 549–610.
[198] M. Moran-Garrido, P. Munoz-Escudero, A. Garcia-Alvarez, I. Garcia-Lunar, C. Barbas, J. Saiz, Optimization of sample extraction and injection-related parameters in HILIC performance for polar metabolite analysis. Application to the study of a model of pulmonary hypertension, J. Chromatogr. A 1685 (2022) 463626.
[199] H.-J. Lee, D.M. Kremer, P. Sajjakulnukit, L. Zhang, C.A. Lyssiotis, A large-scale analysis of targeted metabolomics data from heterogeneous biological samples provides insights into metabolite dynamics, Metabolomics 15 (2019) 103.
[200] W.J. Griffiths, J. Sjovall, Analytical strategies for characterization of bile acid and oxysterol metabolomes, Biochem. Biophys. Res. Commun. 396 (2010) 80–84.
[201] Y. Sekiguchi, N. Mitsuhashi, T. Kokaji, H. Miyakoda, T. Mimura, Development of a comprehensive analytical method for phosphate metabolites in plants by ion chromatography coupled with tandem mass spectrometry, J. Chromatogr. A 1085 (2005) 131–136.
[202] N. Karu, G.W. Dicinoski, M. Hanna-Brown, P.R. Haddad, Determination of pharmaceutically related compounds by suppressed ion chromatography. I. Effects of organic solvent on suppressor performance, J. Chromatogr. A 1218 (2011) 9037–9045.
[203] N. Karu, G.W. Dicinoski, M. Hanna-Brown, K. Srinivasan, C.A. Pohl, P.R. Haddad, Determination of pharmaceutically related compounds by suppressed ion chromatography. III. Role of electrolytic suppressor design, J. Chromatogr. A 1233 (2012) 71–77.
[204] K. Burgess, D. Creek, P. Dewsbury, K. Cook, M.P. Barrett, Semi-targeted analysis of metabolites using capillary-flow ion chromatography coupled to high-resolution mass spectrometry, Rapid Commun. Mass Spectrom. 25 (2011) 3447–3452.
[205] J. Walsby-Tickle, J. Gannon, I. Hvinden, C. Bardella, M.I. Abboud, A. Nazeer, D. Hauton, E. Pires, T. Cadoux-Hudson, C.J. Schofield, J.S.O. McCullagh, Anion-exchange chromatography mass spectrometry provides extensive coverage of

primary metabolomic pathways revealing altered metabolism in IDH1 mutant cells, Commun. Biol. 3 (2020) 247.
[206] H.F. Kvitvang, K.A. Kristiansen, P. Bruheim, Assessment of capillary anion exchange ion chromatography tandem mass spectrometry for the quantitative profiling of the phosphometabolome and organic acids in biological extracts, J. Chromatogr. A 1370 (2014) 70–79.
[207] M. Schwaiger, E. Rampler, G. Hermann, W. Miklos, W. Berger, G. Koellensperger, Anion-exchange chromatography: a powerful tool for merging targeted and non-targeted metabolomics, Anal. Chem. 89 (2017) 7667–7674.
[208] L. Si-Hung, C. Troyer, T. Causon, S. Hann, Sensitive quantitative analysis of phosphorylated primary metabolites using selective metal oxide enrichment and GC- and IC-MS/MS, Talanta 205 (2019) 120147.
[209] Q.A. Soltow, F.H. Strobel, K.G. Mansfield, L. Wachtman, Y. Park, D.P. Jones, High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome, Metabolomics 9 (2013) S132–S143.
[210] A. Le, J. Mak, T.M. Cowan, Metabolic profiling by Reversed-phase/ion-exchange mass spectrometry, J. Chromatogr. B 1143 (2020) 122072.
[211] M.-X. Chen, Z.-Y. Cal, Y. Jiang, Z.-W. Zhu, Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry, J. Chromatogr. A 1272 (2013) 90–99.
[212] R. Tharakan, D. Tao, C. Ubaida-Mohien, R.R. Dinglasan, D.R. Graham, Integrated microfluidic chip and online SCX separation allows untargeted nanoscale metabolomic and peptidomic profling, J. Proteome Res. 14 (2015) 1621–1626.
[213] J. Weiss, Handbook of Ion Chromatography, Wiley, Germany, 2016, pp. 588–590.
[214] F. Michopoulos, N. Whalley, G. Theodoridis, I.D. Wilson, T.P.J. Dunkley, S. E. Critchlow, Targeted profiling of polar intracellular metabolites using ion-pair-high performance liquid chromatography and -ultra-high-performance liquid chromatography coupled to tandem mass spectrometry: applications to serum, urine and tissue extracts, J. Chromatogr. A 1349 (2014) 60–68.
[215] J.M. Knee, T.Z. Rzezniczak, A. Barsch, K.Z. Guo, T.J.S. Merritt, A novel ion-pairing LC/MS metabolomics protocol for study of a variety of biologically relevant polar metabolites, J. Chromatogr. B 936 (2013) 63–73.
[216] F. Madrid-Gambin, S. Oller, S. Marco, O.J. Pozo, C. Andres-Lacueva, R. Llorach, Quantitative plasma profiling by ^{1}H NMR-based metabolomics: impact of sample pretreatment, Front. Mol. Biosci. 10 (2023) 1125532.
[217] C.E. McHugh, T.L. Flott, C.R. Schooff, Z. Smiley, M.A. Puskarich, D.D. Myers, J. G. Younger, A.E. Jones, K.A. Stringer, Rapid, reproducible, quantifiable NMR metabolomics: methanol and methanol:chloroform precipitation for.removal of macromolecules in serum and whole blood, Metabolites 8 (2018) 93.
[218] K. Sidhom, P.O. Obi, A. Saleem, A review of exosomal isolation methods: is size exclusion chromatography the best option? Int. J. Mol. Sci. 21 (2020) 6466.
[219] D. Veyel, E.M. Sokolowska, J.C. Moreno, S. Kierszniowska, J. Cichon, I. Wojciechowska, M. Luzarowski, M. Kosmacz, J. Szalchetko, M. Gorka, M. Meret, A. Graf, E.H. Meyer, L. Willmitzer, A. Skirycz, PROMIS, global analysis of PROtein-metabolite interactions using size separation in Arabidopsis thaliana, J. Biol. Chem. 293 (2018) 12440–12453.
[220] M. Smolarz, A. Kurczyk, K. Jelonek, J. Zyla, L. Mielanczyk, M. Sitkiewicz, M. Pietrowska, J. Polanska, W. Rzyman, P. Widlak, The lipid composition of serum-derived small extracellular vesicles in participants of a lung cancer screening study, Cancers 13 (2021) 3414.
[221] R. Hayasaka, S. Tabata, M. Hasebe, S. Ikeda, T. Hikita, C. Oneyama, J. Yoshitake, D. Onoshima, K. Takahashi, T. Shibata, K. Uchida, Y. Baba, T. Soga, M. Tomita, A. Hirayama, Metabolomics of small extracellular vesicles derived from isocitrate dehydrogenase 1-mutant HCT116 cells collected by semi-automated size exclusion chromatography, Front. Mol. Biosci. 9 (2023) 1049402.
[222] M. Persicke, J. Plassmeier, H. Neuweger, C. Ruckert, A. Puhler, J. Kalinowski, Size exclusion chromatography - an improved method to harvest Cornyebacterium glutamicum cells for the analysis of cytosolic metabolites, J. Biotechnol. 154 (2011) 171–178.
[223] I. Muckenschnabel, R. Falchetto, L.M. Mayr, I. Filipuzzi, SpeedScreen: label-free liquid chromatography-mass spectrometry-based high-throughput screening for the discovery of orphan protein ligands, Anal. Biochem. 324 (2004) 241–249.
[224] D.S. Hage, J.A. Anguizola, A.J. Jackson, R. Matsuda, E. Papastavros, E. Pfaunmiller, Z. Tong, J. Vargas-Badilla, M.J. Yoo, X. Zheng, Chromatographic analysis of drug interactions in the serum proteome, Anal. Methods 3 (2011) 1449–1460.
[225] D.S. Hage, S.A. Tweed, Recent advances in chromatographic and electrophoretic methods for the study of drug-protein interactions, J. Chromatogr. B 699 (1997) 499–528.
[226] N.M. Cassiano, V.V. Lima, R.V. Oliveira, A.C. De Pietro, Q.B. Cass, Development of restricted-access media supports and their application to the direct analysis of biological fluid samples via high-performance liquid chromatography, Anal. Bioanal. Chem. 384 (2006) 1462–1469.
[227] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS, J. Pharm. Biomed. Anal. 44 (2007) 342–355.
[228] S. Souverain, S. Rudaz, J.L. Veuthey, Restricted access materials, and large particle supports for on-line sample preparation: an attractive approach for biological fluids analysis, J. Chromatogr. B 801 (2004) 141–156.
[229] P. Sadílek, D. Šatínský, P. Solich, Using restricted-access materials and column switching in high-performance liquid chromatography for direct analysis of biologically-active compounds in complex matrices, Trends Anal. Chem. 26 (2007) 375–384.
[230] H.D. de Faria, L.C. de C. Abrão, M.G. Santos, A.F. Barbosa, E.C. Figueiredo, New advances in restricted access materials for sample preparation: a review, Anal. Chim. Acta 959 (2017) 43–65.
[231] G. Hopfgartner, E. Bourgogne, Quantitative high-throughput analysis of drugs in biological matrices by mass spectrometry, Mass Spectrom. Rev. 22 (2003) 195–214.
[232] N. Pluym, G. Gilch, G. Scherer, M. Scherer, Analysis of 18 urinary mercapturic acids by two high-throughput multiplex-LC-MS/MS methods, Anal. Bioanal. Chem. 407 (2015) 5463–5476.
[233] J. Bertram, T. Schettgen, T. Kraus, Isotope-dilution method for the determination of 1-vinyl-2-pyrrolidone-mercapturic acid as a potential human biomarker for 1-vinyl-2-pyrrolidone via online SPE ESI-LC/MS/MS in negative ionization mode, J. Chromatogr. B 1033–1034 (2016) 321–327.
[234] N. Quinete, J. Bertram, M. Reska, J. Lang, T. Kraus, Highly selective and automated online SPE LC-MS3 method for determination of cortisol and cortisone in human hair as a biomarker for stress-related diseases, Talanta 134 (2015) 310–316.
[235] Y.D.R. Brunetto, S. Clavijo, Y. Delgado, W. Orozco, M. Gallignani, C. Ayala, V. Cerda, Development of a MSFIA sample treatment system as the front end of GC-MS for atenolol and propranolol determination in human plasma, Talanta 132 (2015) 15–22.
[236] V.M.F. Gonçalves, P. Rodrigues, C. Ribeiro, M.E. Tiritan, Quantification of alprenolol and propranolol in human plasma using a two-dimensional liquid chromatography (2D-LC), J. Pharm. Biomed. Anal. 141 (2017) 1–8.
[237] M. Zobel, K. Klotz, T. Göen, LC-MS/MS procedure for the simultaneous determination of N-acetyl-S-(1-naphthyl)cysteine and N-acetyl-S-(2-naphthyl) cysteine in human urine, J. Chromatogr. B 1074–1075 (2017) 139–145.
[238] J.T. Wu, H. Zeng, Y. Deng, S.E. Unger, High-speed liquid chromatography/tandem mass spectrometry using a monolithic column for high-throughput bioanalysis, Rapid Commun. Mass Spectrom. 15 (2001) 1113–1119.
[239] M. Jemal, Y.Q. Xia, D.B. Whigan, The use of high-flow high-performance liquid chromatography coupled with positive and negative ion electrospray tandem mass spectrometry for quantitative bioanalysis via direct injection of the plasma/serum samples, Rapid Commun. Mass Spectrom. 12 (1998) 1389–1399.
[240] L. Couchman, Turbulent flow chromatography in bioanalysis: a review, Biomed. Chromatogr. 26 (2012) 892–905.
[241] F. Michopoulos, A.M. Edge, G. Theodoridis, I.D. Wilson, Application of turbulent flow chromatography to the metabonomic analysis of human plasma: comparison with protein precipitation, J. Sep. Sci. 33 (2010) 1472–1479.
[242] Y. Mao, M.Q. Huang, Y.Q. Xia, M. Jemal, High-throughput quantitation of nefazodone and its metabolites in human plasma by high flow direct-injection LC-MS/MS, J. Pharm. Biomed. Anal. 43 (2007) 1808–1819.
[243] H.K. Lim, K.W. Chan, S. Sisenwine, J.A. Scatina, Simultaneous screen for microsomal stability and metabolite profile by direct injection turbulent-laminar flow LC-LC and automated tandem mass spectrometry, Anal. Chem. 73 (2001) 2140–2146.
[244] J.L. Herman, Generic method for on-line extraction of drug substances in the presence of biological matrices using turbulent flow chromatography, Rapid Commun. Mass Spectrom. 16 (2002) 421–426.
[245] R. López-Serna, M. Petrović, D. Barceló, Direct analysis of pharmaceuticals, their metabolites, and transformation products in environmental waters using on-line TurboFlow™ chromatography-liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1252 (2012) 115–129.
[246] L. Du, R.L. White, Reducing glycerophosphocholine lipid matrix interference effects in biological fluid assays by using high-turbulence liquid chromatography, Rapid Commun. Mass Spectrom. 22 (2008) 3362–3370.
[247] S. Zhou, H. Zhou, M. Larson, D.L. Miller, D. Mao, X. Jiang, W. Naidong, High-throughput biological sample analysis using on-line turbulent flow extraction combined with monolithic column liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 19 (2005) 2144–2150.
[248] D.S. Hage (Ed.), Handbook of Affinity Chromatography, 2nd Ed., CRC Press, Boca Raton, 2006.
[249] S. Iftekhar, S.T. Ovbude, D.S. Hage, Affinity-based methods for the analysis of emerging contaminants in wastewater and related samples, in: M. Kumar, D. D. Snow, R. Honda, S. Mukherjee (Eds.), Contaminants in Drinking and Wastewater Sources: Challenges and Reigning Technologies, Singapore, Springer, 2021, pp. 37–64.
[250] A.C. Moser, D.S. Hage, Immunoaffinity chromatography: an introduction to applications and recent development, Bioanalysis 2 (2011) 769–790.
[251] L. Zhang, X. Dou, W. Kong, C. Liu, X. Han, M. Yang, Assessment of critical points and development of a practical strategy to extend the applicable scope of immunoaffinity column cleanup for aflatoxin detection in medicinal herbs, J. Chromatogr. A 1483 (2017) 56–63.
[252] E. Calleri, G. Marrubini, G. Brusotti, G. Massolini, G. Caccialanza, Development and integration of an immunoaffinity monolithic disk for the online solid-phase extraction and HPLC determination with fluorescence detection of aflatoxin B1 in aqueous solutions, J. Pharm. Biomed. Anal. 44 (2007) 396–403.
[253] F. Berthiller, C. Brera, C. Crews, M.H. Iha, R. Krsha, V.M.T. Lattanzio, S. MacDonald, R.J. Malone, M. Solfrizzo, J. Stroka, T.B. Whittaker, Developments in mycotoxin analysis: an update for 2013–2014, World Mycotoxin J. 8 (2015) 5–36.
[254] Y. Zhu, Y.I. Hassan, S. Shao, T. Zhou, Employing immuno-affinity for the analysis of various microbial metabolites of the mycotoxin deoxynivalenol, J. Chromatogr. A 1556 (2018) 81–87.

[255] Y. Wang, Y. Xu, X. Zhang, E. Wang, Y. Dong, Preparation of an immunoaffinity column and its application in sample cleanup for methandrostenolone residues detection, J. Chromatogr. B 879 (2011) 2149–2154.
[256] A. Desmarchelier, S. Tessiot, T. Bessaire, L. Racault, E. Fiorese, A. Urbani, W. Chan, P. Cheng, P. Mottier, Combining the quick, easy, cheap, effective, rugged, and safe approach and clean-up by immunoaffinity column for the analysis of 15 mycotoxins by isotope dilution liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1337 (2014) 75–84.
[257] V.M.T. Lattanzio, B. Ciasca, S. Powers, A. Visconti, Improved method for the simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in cereals and derived products by liquid chromatography-tandem mass spectrometry after multi-toxin immunoaffinity clean up, J. Chromatogr. A 1354 (2014) 139–143.
[258] J. Oklestkova, D. Tarkowska, L. Eyer, T. Elbert, A. Marek, Z. Smrzova, O. Novak, M. Franek, V.N. Zbabinskii, M. Strnad, Immunoaffinity chromatography combined with tandem mass spectrometry: a new tool for the selective capture and analysis of brassinosteroid plant hormones, Talanta 170 (2017) 432–440.
[259] J. Cai, J. Henion, On-line immunoaffinity extraction-coupled column capillary liquid chromatography/tandem mass spectrometry: trace analysis of LSD analogs and metabolites in human urine, Anal. Chem. 68 (1996) 72–78.
[260] S. Feng, M.A. ElSohly, S. Salamone, M.Y. Salem, Simultaneous analysis of Δ9-THC and its major metabolites in urine, plasma, and meconium by GC-MS using an immunoaffinity extraction procedure, J. Anal. Toxicol. 24 (2000) 395–402.
[261] J. Xie, T. Peng, D.D. Cheng, Q.J. Zhang, G.M. Wang, X. Guo, F. Jiang, D. Cheng, J. Deng, Determination of malachite green, crystal violet, and their leuco-metabolites in fish by HPLC-VIS detection after immunoaffinity column clean-up, J. Chromatogr. B 913–914 (2012) 123–128.
[262] T. Higashi, K. Mitamura, H. Ohmi, N. Yamada, K. Shimada, K. Tanaka, H. Honjo, Levels of 24,25-dihydroxyvitamin D_3, 25-hydrovitamin D_3 and 25-hydrovitamin D_3-3-sulphate in human plasma, Ann. Clin. Biochem. 36 (1999) 43–47.
[263] X. Zhang, D. Zhang, K. He, Combining an effective immuno-affinity column with ELISA for reliable and visual detection of furaltadone metabolites in aquatic products, Anal. Methods 11 (2019) 1270–1275.
[264] D.H. Thomas, M. Beck-Westermeyer, D.S. Hage, Determination of atrazine in water using high-performance immunoaffinity chromatography and reversed-phase liquid chromatography, Anal. Chem. 66 (1994) 3823–3829.
[265] J.G. Rollag, M. Beck-Westermeyer, D.S. Hage, Analysis of pesticide degradation products by tandem high-performance immunoaffinity chromatography and reversed-phase liquid chromatography, Anal. Chem. 68 (1996) 3631–3637.
[266] M.A. Nelson, A. Gates, M. Dodlinger, D.S. Hage, Development of a portable immunoextraction/RPLC system for field studies of herbicide residues, Anal. Chem. 76 (2004) 805–813.
[267] M.A. Nelson, E. Papastavros, M. Dodlinger, D.S. Hage, Environmental analysis by on-line immunoextraction and reversed-phase liquid chromatography: optimization of the immunoextraction/RPLC interface, J. Agric. Food Chem. 55 (2007) 3788–3797.
[268] X. Li, T.A. Gianoulis, K.Y. Yip, M. Gerstein, M. Snyder, Extensive in vivo metabolite-protein interactions revealed by large-scale systematic analyses, Cell 143 (2010) 639–650.
[269] D.S. Hage, Affinity chromatography: a review of clinical applications, Clin. Chem. 45 (1999) 593–615.
[270] D.S. Hage, High performance affinity chromatography: a powerful tool for studying serum protein binding, J. Chromatogr. B 768 (2002) 3–30.
[271] J. Anguizola, K.S. Joseph, O.S. Barnaby, R. Matsuda, G. Alvarado, W. Clarke, R. L. Cerny, D.S. Hage, Development of affinity microcolumns for drug–protein binding studies in personalized medicine: interactions of sulfonylurea drugs with in vivo glycated human serum albumin, Anal. Chem. 85 (2013) 4453–4460.
[272] B. Loun, D.S. Hage, Characterization of thyroxine-albumin binding using high-performance affinity chromatography: I. Interactions at the warfarin and indole sites of albumin, J. Chromatogr. B 579 (1992) 225–235.
[273] B. Loun, D.S. Hage, Characterization of thyroxine-albumin binding using high-performance affinity chromatography: II. Comparison of the binding of thyroxine, triiodothyronines and related compounds at the warfarin and indoles sites of human serum albumin, J. Chromatogr. B 665 (1995) 303–314.
[274] C.M. Ohnmacht, S. Chen, Z. Tong, D.S. Hage, Studies by biointeraction chromatography of binding by phenytoin metabolites to human serum albumin, J. Chromatogr. B 836 (2006) 83–91.
[275] J. Chen, C. Ohnmacht, D.S. Hage, Studies of phenytoin binding to human serum albumin by high-performance affinity chromatography, J. Chromatogr. B 809 (2004) 137–145.
[276] K.S. Joseph, A.C. Moser, S. Basiaga, J.E. Schiel, D.S. Hage, Evaluation of alternatives to warfarin as probes for Sudlow site I of human serum albumin: characterization by high performance affinity chromatography, J. Chromatogr. A 1216 (2009) 3492–3500.
[277] M.L. Conrad, A.C. Moser, D.S. Hage, Evaluation of indole-based probes for studying drug binding to human serum albumin in high-performance affinity separations, J. Sep. Sci. 32 (2009) 1145–1155.
[278] Z. Tong, D.S. Hage, Characterization of interaction kinetics between chiral solutes and human serum albumin by using high-performance affinity chromatography and peak profiling, J. Chromatogr. A 1218 (2011) 6892–6897.
[279] Ö.C. Zeki, C.C. Eylem, T. Reçber, S. Kır, E. Nemutlu, Integration of GC–MS and LC-MS for untargeted metabolomics profiling, J. Pharm. Biomed. Anal. 190 (2020) 113509.
[280] A.T. James, A.J.P. Martin, Gas-liquid partition chromatography. A technique for the analysis of volatile materials, Analyst 77 (1952) 915–932.
[281] O. Fiehn, Metabolomics by gas chromatography-mass spectrometry: the combination of targeted and untargeted profiling, Curr. Protoc. Mol. Biol. 7 (2017) 232–235.
[282] H. Lu, Y. Liang, W.B. Dunn, H. Shen, D.B. Kell, Comparative evaluation of software for deconvolution of metabolomics data based on GC-TOF-MS, Trends Anal. Chem. 27 (2008) 215–227.
[283] A. Mastrangelo, A. Ferrarini, F. Rey-Stolle, A. García, C. Barbas, From sample treatment to biomarker discovery: a tutorial for untargeted metabolomics based on GC-(EI)-Q-MS, Anal. Chim. Acta 900 (2015) 21–35.
[284] W.B. Dunn, D. Broadhurst, D.I. Ellis, M. Brown, A. Halsall, S.O. Hagan, I. Spasic, A. Tseng, D.B. Kell, A GC-TOF-MS study of the stability of serum and urine metabolomes during the UK Biobank sample collection and preparation protocols, Int. J. Epidemiol. 37 (2008) 23–30.
[285] P. Begley, S. Francis-Mclyntre, W.B. Dunn, D.I. Broadhurst, A. Halsall, A. Tseng, J. Knowles, H. Consortium, R. Goodacre, D.B. Kell, Development and performance of a gas chromatography-time-of-flight mass spectrometry analysis for large-scale nontargeted metabolomic studies of human serum, Anal. Chem. 81 (2009) 7038–7046.
[286] T. Kind, V. Tolstikov, O. Fiehn, R.H. Weiss, A comprehensive urinary metabolomic approach for identifying kidney cancer, Anal. Biochem. 363 (2007) 185–195.
[287] M.M. Koek, B. Muilwijk, M.J. Van Der Werf, T. Hankemeier, Microbial metabolomics with gas chromatography/mass spectrometry, Anal. Chem. 78 (2006) 1272–1281.
[288] J.L. Little, Artifacts in trimethylsilyl derivatization reactions and ways to avoid them, J. Chromatogr. A 844 (1999) 1–22.
[289] N. Schauer, A.R. Fernie, Plant metabolomics: towards biological function and mechanism, Trends Plant Sci. 11 (2006) 508–516.
[290] Y. Wang, L. Xu, H. Shen, J. Wang, W. Liu, X. Zhu, R. Wang, X. Sun, L, Liu, Metabolomic analysis with GC-MS to reveal potential metabolites and biological pathways involved in Pb & Cd stress response of radish roots, Sci. Rep. 5 (2015) 1–13.
[291] O. Begou, H.G. Gika, I.D. Wilson, G. Theodoridis, Hyphenated MS-based targeted approaches in metabolomics, Analyst 142 (2017) 3079–3100.
[292] W.B. Dunn, D.I. Ellis, Metabolomics: current analytical platforms and methodologies, Trends Anal. Chem. 24 (2005) 285–294.
[293] O. Fiehn, J. Kopka, P. Dörmann, T. Altmann, R.N. Trethewey, L. Willmitzer, Metabolite profiling for plant functional genomics, Nat. Biotechnol. 18 (2000) 1157–1161.
[294] Y. Tikunov, A. Lommen, C.H. Ric de Vos, H.A. Verhoeven, R.J. Bino, R.D. Hall, A. G. Bovy, A novel approach for nontargeted data analysis for metabolomics. Large-Scale Profiling of Tomato Fruit Volatiles, Plant Physiol. 139 (2005) 1125–1137.
[295] U. Roessner, C. Wagner, J. Kopka, R.N. Trethewey, L. Willmitzer, Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry, Plant J. 23 (2000) 131–142.
[296] U. Roessner-Tunali, B. Hegemann, A. Lytovchenko, F. Carrari, C. Bruedigam, D. Granot, A.R. Fernie, Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development, Plant Physiol. 133 (2003) 84–99.
[297] J.C. Verdonk, C.H.R. De Vos, H.A. Verhoeven, M.A. Haring, A.J. Van Tunen, R. C. Schuurink, Regulation of floral scent production in petunia revealed by targeted metabolomics, Phytochemistry 62 (2003) 997–1008.
[298] A. Jiye, J. Trygg, J. Gullberg, A.I. Johansson, H. Antti, S.L. Marklund, T. Moritz, Extraction and GC/MS analysis of the human blood plasma metabolome, Anal. Chem. 77 (2005) 8086–8094.
[299] O. Fiehn, T. Kind, Metabolite profiling in blood plasma, in: W. Weckwert (Ed.), Metabolomics, Humana Press, Totowa, Methods in Molecular Biology, 2007, pp. 3–17.
[300] S. Nakamizo, T. Sasayama, M. Shinohara, Y. Irino, S. Nishiumi, M. Nishihara, H. Tanaka, K. Tanaka, K. Mizukawa, T. Itoh, M. Taniguchi, K. Hosoda, M. Yoshida, E. Kohmura, GC/MS-based metabolomic analysis of cerebrospinal fluid (CSF) from glioma patients, J. Neurooncol. 113 (2013) 65–74.
[301] M. Chen, M. Shen, Y. Li, C. Liu, K. Zhou, W. Hu, B. Xu, Y. Xia, W. Tang, GC-MS-based metabolomic analysis of human papillary thyroid carcinoma tissue, Int. J. Mol. Med. 36 (2015) 1607–1614.
[302] G. Desbrosses, D. Steinhauser, J. Kopka, M. Udvardi, Metabolome analysis using GC-MS, in: A.J. Márquez (Ed.), Lotus Japonicus Handbook, Springer, Netherlands, 2005, pp. 165–174.
[303] K. Hiller, J. Hangebrauk, C. Jäger, J. Spura, K. Schreiber, D. Schomburg, Metabolite detector: Comprehensive analysis tool for targeted and nontargeted GC/MS based metabolome analysis, Anal. Chem. 81 (2009) 3429–3439.
[304] S.P. Putri, M.M.M. Ikram, A. Sato, H.A. Dahlan, D. Rahmawati, Y. Ohto, E. Fukusaki, Application of gas chromatography-mass spectrometry-based metabolomics in food science and technology, J. Biosci. Bioeng. 133 (2022) 425–435.
[305] D.J. Beale, F.R. Pinu, K.A. Kouremenos, M.M. Poojary, V.K. Narayana, B. A. Boughton, K. Kanojia, S. Dayalan, O.A.H. Jones, D.A. Dias, Review of recent developments in GC-MS approaches to metabolomics-based research, Metabolomics 14 (2018) 152.
[306] C. Fang, H. Wang, Z. Lin, X. Liu, L. Dong, T. Jiang, Y. Tan, Z. Ning, Y. Ye, G. Tan, G. Xu, Metabolic reprogramming and risk stratification of hepatocellular carcinoma studied by using gas chromatography–mass spectrometry-based metabolomics, Cancers 14 (2022) 231.
[307] M.G. Jajin, R. Abooshahab, K. Hooshmand, A. Moradi, S.D. Siadat, R. Mirzazadeh, K.G. Chegini, M. Hedayati, Gas chromatography-mass spectrometry-based

untargeted metabolomics reveals metabolic perturbations in medullary thyroid carcinoma, Sci. Reports 12 (2022) 8397.
[308] N. Di Giovanni, M.A. Meuwis, E. Louis, J.F. Focant, Correlations for untargeted GC × GC-HRTOF-MS metabolomics of colorectal cancer, Metabolomics 19 (2023) 85.
[309] G.H.B. Durate, A.M.A. de Piloto, A.A.R. Silva, H.R. Zomara-Obando, A.G. Amaral, A. de Sousa Mesquita, J. Schmidt-Filho, V.C.C. de Lima, F.D. Costa, V.P. Andrade, A.M. Porcari, M.N. Eberlin, A.V.C. Siminato, Gas chromatography-mass spectrometry untargeted profiling of non-Hodgkin's lymphoma urinary metabolite markers, Anal. Bioanal. Chem. 412 (2020) 7469–7480.
[310] H. Xin, W.Q. Wang, Z.W. Yang, J.X. Liu, S. Li, L. Wang, Q.L. Jiang, L.L. Jia, Metabolomics studies of prostate cancer using gas chromatography-mass spectrometry, Trans, Cancer Res. 5 (2016) 302–314.
[311] M.M. Koek, R.H. Jellema, J. van der Greef, A.C. Tas, T. Hankemeier, Quantitative metabolomics based on gas chromatography mass spectrometry: status and perspectives, Metabolomics 7 (2011) 307–328.
[312] M.F. Almstetter, P.J. Oefner, K. Dettmer, Comprehensive two-dimensional gas chromatography in metabolomics, Anal. Bioanal. Chem. 402 (2012) 1993–2013.
[313] Y. Li, W. Leng, J. Xue, L. Yuan, H. Liu, R. Gao, A multi-omics-based investigation into the flavor formation mechanisms during the fermentation of traditional Chinese shrimp paste, Food Res. Intl. 166 (2023) 112585.
[314] X. Fang, Y. Liu, J. Xiao, C. Ma, Y. Huang, GC–MS and LC-MS/MS metabolomics revealed dynamic changes of volatile and non-volatile compounds during withering process of black tea, Food Chem. 410 (2023) 135396.
[315] Q. Zhao, J. Xi, D. Xu, Y. Jin, F. Wu, Q. Tong, Y. Yin, X. Xu, A comparative HS-SPME/GC-MS-based metabolomics approach for discriminating selected japonica rice varieties from different regions of China in raw and cooked form, Food Chem. 385 (2022) 132701.
[316] X. Song, S. Jing, L. Zhu, C. Ma, T. Song, J. Wu, Q. Zhao, F. Zheng, M. Zhao, F. Chen, Untargeted and targeted metabolomics strategy for the classification of strong aroma-type baijiu (liquor) according to geographical origin using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry, Food Chem. 314 (2020) 126098.
[317] A. Tarafder, Metamorphosis of supercritical fluid chromatography to SFC: an overview, Trends Anal. Chem. 81 (2016) 3–10.
[318] V. Desfontaine, D. Guillarme, E. Francotte, L. Nováková, Supercritical fluid chromatography in pharmaceutical analysis, J. Pharm. Biomed. Anal. 113 (2015) 56–71.
[319] A. Periat, A. Grand-Guillaume Perrenoud, D. Guillarme, Evaluation of various chromatographic approaches for the retention of hydrophilic compounds and MS compatibility, J. Sep. Sci. 36 (2013) 3141–3151.
[320] A. Grand-Guillaume Perrenoud, J. Boccard, J.L. Veuthey, D. Guillarme, Analysis of basic compounds by supercritical fluid chromatography: attempts to improve peak shape and maintain mass spectrometry compatibility, J. Chromatogr. A (2012, 1262,) 205–213.
[321] D. Guillarme, V. Desfontaine, S. Heinisch, J.L. Veuthey, What are the current solutions for interfacing supercritical fluid chromatography and mass spectrometry? J. Chromatogr. B 1083 (2018) 160–170.
[322] D. Wolrab, M. Chocholoušková, R. Jirásko, O. Peterka, M. Holčapek, Validation of lipidomic analysis of human plasma and serum by supercritical fluid chromatography-mass spectrometry and hydrophilic interaction liquid chromatography-mass spectrometry, Anal. Bioanal. Chem. 412 (2020) 2375–2388.
[323] A. Sen, M.R. Lewis, R.S. Plumb, I.D. Wilson, J.K. Nicholson, N.W. Smith, Analysis of polar urinary metabolites for metabolic phenotyping using supercritical fluid chromatography and mass spectrometry, J. Chromatogr. A 1449 (2016) 141–155.
[324] R.S. Plumb, L.A. Gethings, P.D. Rainville, G. Isaac, R. Tengove, A.M. King, I. D. Wilson, Advances in high throughput LC/MS based metabolomics: a review, Trends Anal. Chem. 160 (2023) 116954.
[325] T.O. Metz, J.S. Page, E.S. Baker, K. Tang, J. Ding, Y. Shen, R.D. Smith, High-resolution separations and improved ion production and transmission in metabolomics, Trends Anal. Chem. 27 (2008) 205–214.
[326] J.J. Heijnen, Impact of thermodynamic principles in systems biology, Adv. Biochem. Eng. Biotechnol. 121 (2010) 139–162.
[327] M. Stitt, J. Lunn, B. Usadel, Arapidopsis and primary photosynthetic metabolism - more than the icing on the cake, Plant J. 61 (2010) 1067–1091.
[328] A.B. Canelas, A. ten Pierick, C. Ras, R.M. Seifar, J.C. van Dam, W.M. van Gulik, J. J. Heijnen, Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolites, Anal. Chem. 81 (2009) 7379–7389.
[329] P.J. Taylor, Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry, Clin. Biochem. 38 (2005) 328–334.
[330] J. Draper, A.J. Lloyd, R. Goodacre, M. Beckmann, Flow infusion electrospray ionisation mass spectrometry for high throughput, non-targeted metabolite fingerprinting: a review, Metabolomics 9 (2018) 4–29.
[331] H.E. Roth, R. Powers, Meta-analysis reveals both the promises and the challenges of clinical metabolomics, Cancers 14 (2022) 3392.
[332] F. Bhinderwala, T. Vu, T.G. Smith, J. Kosacki, D.D. Marshall, Y. Xu, M. Morton, R. Powers, Leveraging the HMBC to facilitate metabolite identification, Anal. Chem. 94 (2022) 16308–16318.
[333] M.J. Jeppesen, R. Powers, Multiplatform untargeted metabolomics, Magn. Reson. Chem. 1–26 (2023).
[334] D.-W. Li, A. Leggett, L. Bruschweiler-Li, R. Bruschweiler, COLMARq: A web server for 2D NMR peak picking and quantitative comparative analysis of cohorts of metabolomics samples, Anal. Chem. 94 (2022) 8674–8682.
[335] C. Wang, I. Timari, B. Zhang, D.-W. Li, A. Leggett, A.O. Amer, L. Bruschweiler-Li, R.E. Kopec, R. Bruschweiler, COLMAR lipids web server and ultrahigh-resolution methods for two-dimensional nuclear magnetic resonance- and mass spectrometry-based lipidomics, J. Proteome Res. 19 (2020) 1674–1683.
[336] A. Leggett, C. Wang, D.-W. Li, A. Somogyi, L. Bruschweiler-Li, R. Bruschweiler, Identification of unknown metabolomics mixture compounds by combinding NMR, MS, and cheminformatics, Methods Enzymol. 615 (2019) 407–422.
[337] A.K. Shukla, J.H. Futrell, Tandem mass spectrometry: dissociation of ions by collisional activation, J. Mass Spectrom. 35 (2000) 1069–1090.
[338] K.R. Clauser, P. Baker, A.L. Burlingame, Role of accurate mass measurement (±10 ppm) in protein identification strategies employing MS or MS/MS and database searching, Anal. Chem. 71 (1999) 2871–2882.
[339] F. Bhinderwala, S. Lonergan, J. Woods, C. Zhou, P.D. Fey, R. Powers, Expanding the coverage of the metabolome with nitrogen-based NMR, Anal. Chem. 90 (2018) 4521–4528.
[340] F. Bhinderwala, P. Evans, K. Jones, B.R. Laws, T.G. Smith, M. Morton, R. Powers, Phosphorus NMR and its application to metabolomics, Anal. Chem. 92 (2020) 9536–9545.
[341] T. von Schlippenbach, P.J. Oefner, W. Gronwald, Systematic evaluation of non-uniform sampling parameters in the targeted analysis of urine metabolites by ^{1}H, ^{1}H 2D NMR spectroscopy, Sci. Reports 8 (2018) 4249.
[342] B. Zhang, R. Powers, E.M. O'Day, Evaluation of non-uniform sampling 2D 1H–13C HSQC spectra for semi-quantitative metabolomics, Metabolites 10 (2020) 203.
[343] J. Mauhart, S. Glanzer, P. Sakhaii, W. Bermel, K. Zangger, Faster and cleaner real-time pure shift NMR experiments, J. Magn. Reson. 259 (2015) 207–215.
[344] D. Schulze-Sunninghausen, J. Becker, M.R.M. Koos, B. Luy, Improvements, extensions, and practical aspects of rapid ASAP-HSQC and ALSOFAST-HSQC pulse sequences for studying small molecules at natural abundance, J. Magn. Reson. 281 (2017) 151–161.
[345] S. Ghosh, A. Sengupta, K. Chandra, SOFAST-HMQC - an efficient tool for metabolomics, Anal. Bioanal. Chem. 409 (2017) 6731–6738.
[346] D.M. Jacobs, L. Spiesser, M. Garnier, N. de Roo, F. van Dorsten, B. Hollebrands, E. van Velzen, R. Draijer, J. van Duynhoven, SPE–NMR metabolite sub-profiling of urine, Anal. Bioanal. Chem. 404 (2012) 2349–2361.
[347] S. Rezzi, F.A. Vera, F.-P.-J. Martin, S. Wang, D. Lawler, S. Kochhar, Automated SPE-RP-HPLC fractionation of biofluids combined to off-line NMR spectroscopy for biomarker identification in metabolomics, J. Chromatogr. B 871 (2008) 271–278.
[348] Z. Miao, M. Jin, X. Liu, W. Guo, X. Jin, H. Liu, Y. Wang, The application of HPLC and microprobe NMR spectroscopy in the identification of metabolites in complex biological matrices, Anal. Bioanal. Chem. 407 (2015) 3405–3416.
[349] R. Hammerl, O. Frank, T. Hofmann, Differential off-line LC-NMR (DOLC-NMR) metabolomics to monitor tyrosine-induced metabolome alterations in Saccharomyces cerevisiae, J. Agric. Food Chem. 65 (2017) 3230–3241.
[350] R.L. Haner, P.A. Keifer, Flow probes for NMR spectroscopy, in: S. Ashbrook (Ed.), Encyclopedia of Magnetic Resonance: Wiley, Hoboken, 2009, pp. 1–11.
[351] J. Bart, A.J. Kolkman, A.J. Oosthoek-de Vries, K. Koch, P.J. Nieuwland, H.J.W. G. Janssen, J.P.J.M. van Bentum, K.A.M. Ampt, F.P.J.T. Rutjes, S.S. Wijmenga, H. J.G.E. Gardeniers, A.P.M. Kentgens, A microfluidic high-resolution NMR flow probe, J. Am. Chem. Soc. 131 (2009) 5014–5015.
[352] M.W. Voehler, G. Collier, J.K. Young, M.P. Stone, M.W. Germann, Performance of cryogenic probes as a function of ionic strength and sample tube geometry, J. Magn. Reson. 183 (2006) 102–109.
[353] D. Feng, T. Xu, H. Li, X. Shi, G. Xu, Single-cell metabolomics analysis by microfluidics and mass spectrometry: recent new advances, J. Anal. Testing 4 (2020) 198–209.
[354] M. He, Y. Zhou, W. Cui, Y. Yang, H. Zhang, X. Chen, W. Pang, X. Duan, An on-demand femtoliter droplet dispensing system based on a gigahertz acoustic resonator, Lab Chip 18 (2018) 2540–2546.
[355] D. Foresti, K.T. Kroll, R. Amissah, F. Sillani, K.A. Homan, D. Poulikakos, J. A. Lewis, Acoustophoretic printing, Sci Adv. 4 (2018) 9.
[356] F. Chen, Y. Zhang, Y. Nakagawa, H. Zeng, C. Luo, H. Nakajima, K. Uchiyama, J. M. Lin, A piezoelectric drop-on-demand generator for accurate samples in capillary electrophoresis, Talanta 107 (2013) 111–117.
[357] R.D. Pedde, H. Li, C.H. Borchers, M. Akbari, Microfluidic-mass spectrometry interfaces for translational proteomics, Trends Biotechnol. 35 (2017) 954–970.
[358] D.A.V. Medina, E.V.S. Maciel, F.M. Lancas, Miniaturization of liquid chromatography coupled to mass spectrometry. 3. Achievements on chip-based LC-MS devices, Trends Anal. Chem. 131 (2020) 116003.
[359] P. Cui, S. Wang, Application of microfluidic chip technology in pharmaceutical analysis: a review, J. Pharmaceut. Anal. 9 (2019) 238–247.