Journal of Analysis and Testing
https://doi.org/10.1007/s41664-020-00126-z

REVIEW

Challenges in Analysis of Hydrophilic Metabolites Using Chromatography Coupled with Mass Spectrometry

Qingyu Hu[1,2,3] · Huiru Tang[3] · Yulan Wang[4]

Received: 2 February 2020 / Accepted: 26 March 2020

Abstract

Hydrophilic metabolites play important roles in cellular energy metabolism, signal transduction, immunity. However, there are challenges in both identification and quantification of the hydrophilic metabolites due to their weak interactions with C18-reversed-phase liquid chromatography (RPLC), leading to poor retention of hydrophilic metabolites on the columns. Many strategies have been put forward to increase the retention behavior of hydrophilic metabolites in the RPLC system. Non-derivatization methods are mainly focused on the development of new chromatographic techniques with different separation mechanisms, such as capillary electrophoresis, ion-pairing RPLC etc. Derivatization methods improve the hydrophobicity of metabolites and can enhance the MS response. This review mainly focused on the illustration of challenges of LCMS in the analysis of hydrophilic metabolomics field, and summarized the non-derivatization and derivatization strategies, with the intention of providing multiple choices for analysis of hydrophilic metabolites.

Keywords Hydrophilic metabolites · Hydrophilic interaction chromatography · Ion-pairing reversed-phase liquid chromatography · Ion chromatography · Capillary electrophoresis · Derivatization

Abbreviations

ATP	Adenosine triphosphate
BSTFA	N, *O*-bis (trimethylsilyl) trifluoroacetamide
cAMP	Cyclic adenosine monophosphate
CapIC	Capillary ion chromatography
CE	Capillary electrophoresis
cGMP	Cyclic guanosine monophosphate
dCMP	Deoxycytidine monophosphate
dCDP	Deoxycytidine diphosphate
dCTP	Deoxycytidine triphosphate
DIPEA	Diisopropylethylamine
EDC	1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide
EOF	Electro-osmotic flow
ESI	Electrospray ionization
GC–MS	Gas chromatography–mass spectrometry
GDP	Guanosine diphosphate
GPCR	G protein-coupled receptor.
GTP	Guanosine triphosphate
HAc	Acetic acid
HFIP	Hexafluoroisopropanol
HILIC	Hydrophilic interaction liquid chromatography
HMDB	Human Metabolome Database Bank
HMDS	Hexamethyldisilazane
HXA	Hexylamine
IC	Ion chromatography
IP(RP)LC	Ion-pairing(reversed-phase) chromatography
IPR	Ion pairing reagents
LOD	Limit of detection
LOQ	Limit of quantification

✉ Yulan Wang
yulan.wang@ntu.edu.sg

[1] CAS Key Laboratory of Magnetic Resonance in Biological Systems, State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, National Centre for Magnetic Resonance in Wuhan, Wuhan Institute of Physics and Mathematics, Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences (CAS), Wuhan 430071, People's Republic of China

[2] University of Chinese Academy of Sciences, Beijing 10049, People's Republic of China

[3] State Key Laboratory of Genetic Engineering, Zhongshan Hospital and School of Life Sciences, Laboratory of Metabonomics and Systems Biology, Human Phenome Institute, Fudan University, Shanghai 200433, People's Republic of China

[4] Singapore Phenome Center, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 639798, Singapore

Published online: 13 April 2020

MS	Mass spectrometry
NaADP	Niacin adenosine dinucleotide phosphate
NAD+	Oxidized form of nicotinamide-adenine dinucleotide
NADH	Reduced form of nicotinamide-adenine dinucleotide
NADP+	Oxidized form of nicotinamide-adenine dinucleotide phosphate
NADPH	Reduced form of nicotinamide-adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NPLC	Normal phase liquid chromatography
RPLC	Reversed-phase liquid chromatography
TBA	Tributylamine
TCA	Tricarboxylic acids
TEA	Trimethylamine
TMCS	Trimethylchlorosilane
4-APEBA	4-(2-{[2-(4-Bromophenyl)ethyl](dimethyl) ammonio}ethoxy)anilinium dibromide
6-AQC	6-Aminoquinoline-*N*-hydroxysuccinimide ester
5-AIQC	5-Aminoisoquinolyl-*N*-hydroxysuccinimidyl carbamate

1 Introduction

Hydrophilic metabolites are widely present in biological samples, which cover many important metabolic pathways, including amino acid metabolism, nucleotides metabolism, central carbon metabolism, water-soluble vitamins, and cofactors metabolism and so on. Therefore, it is not an understatement that hydrophilic metabolites infiltrate at least half of metabolic pathways. In addition, fluxomics, an emerging strategy in recent years to depict the flow rate of metabolic pathways, are mainly concerned with the central carbon metabolism [1, 2], which are also hydrophilic metabolites. Therefore, precise identification and quantification of hydrophilic metabolites are important in wide biological research.

Although the hydrophilic metabolites play important roles in physiological and pathological processes, it is very difficult to measure these highly hydrophilic, polar or even ionic metabolites. Nuclear magnetic resonance (NMR) is one of the first tools employed for polar metabolite analysis because it does not require separation and complex pretreatment [3, 4]. However, hydrophilic metabolites in biological fluids present a fairly wide dynamic range from nM to mM [5, 6]. Although NMR is very useful for the identification and quantification of metabolites with great reproducibility, it can only detect those metabolites with high concentrations such as amino acids, organic acids (above 100 μM in general). NMR lacks sufficient sensitivity for low abundance metabolites, like phosphorylated metabolites, hormones, and cofactors. In addition, proton NMR spectra display heavy peak overlapping, likely leading to inaccurate concentration calculation. Although the highly sensitive chromatography coupled with mass spectrometry could overcome these issues, the weak retention behavior and ion suppression pose great challenges in LC–MS analysis.

In the literature, most reviews focused on the specific technologies [7–9] or the full metabolomics and lipidomics [10, 11]. In this review, we pay more attention to the hydrophilic metabolites with important biological functions and discuss the challenge of LC–MS analysis for the hydrophilic metabolites. The development of derivatization strategies with high-retention in LC and high-sensitivity in MS detection should have huge potential for detecting the hydrophilic metabolites.

2 The Biological Functions of Hydrophilic Metabolomics

2.1 Amino Acids and Metabolites with Amino Group

Amino acids are zwitterionic metabolites with both amino group and carboxyl group, which can integrate into central carbon metabolism through a range of organic acids intermediates. For example, glutamic acid and glutamine can integrate Krebs cycle through the clawback mechanism producing γ-aminobutyric acid (GABA) [12]. Many researches indicate that glutamic acid is an important substrate for metabolic reprogramming for cancer cells. Tryptophan (Trp) is reported as a signal molecule [13] that participates in regulating immunity [14], neurological function [15, 16] and gut steady-state [17, 18] through the kynurenine pathway. The catabolism of Trp has immune inhibition to Th1 lymphocytes [19]. Kynurenine is regarded as an endodermic vasodilator to regulate the effects of nitric oxide (NO) [20]. Indoleamine-2,3-dioxygenase (IDO) is the first rate-limiting enzyme in the degradation of the kynurenine pathway and is closely related to inflammation inhibition and tolerance [13]. In addition, the Trp also mediates the development of many diseases in the co-metabolism with the intestinal flora via the 5-hydroxytryptamine pathway and mediates the signal transduction of aryl hydrocarbon receptor (AhR) [18]. The β-aminoisobutyric acid produced by catabolism of valine is significantly increased in the urine of patients with bladder tumors [21]. Trimethylamine *N*-oxide (TMAO) is a pro-inflammatory factor in the metabolism of intestinal flora after a high choline diet [22]. Thyroid hormones, norepinephrine, and other organic amine hormones are known to participate in metabolic regulation.

2.2 Organic Carboxyl Acids

Organic carboxylic acids, referring to aliphatic mono- or polycarboxylic acid containing small numbers of carbons (less than 10 carbons), including short-chain fatty acids, keto acids, hydroxyl acids, et al. These metabolites have very weak chromatographic retention behavior due to the presences of a large number of polar ionized groups. Short-chain fatty acids (SCFs) have important physiological functions. For example, butyric acid is considered as an important product of intestinal flora fermentation and plays a key role in mediating host metabolism [23, 24], regulating the immune system and cell proliferation. Polycarboxylic acids in the Krebs cycle such as oxaloacetic acid and citric acid can promote the Warburg effects through metabolic reprogramming in cancer cells. α-ketoglutaric acid plays an essential role in the integrated regulation between cellular carbon metabolism and nitrogen metabolism [25]. Glyoxylic acid is the precursor molecule of oxalic acid which can lead to kidney stones [26]. In addition to energy metabolism, the Krebs cycle also affects immunity [27]. Succinic acid is known as a signaling molecule for macrophages produced in lipopolysaccharide and IFN-γ treated macrophages [28, 29]. He et al. found that succinic acid could bind to a G protein-coupled receptor GPR9 (SUCNR1), producing a therapeutic effect on hypertension by modulating the renin–angiotensin system [30]. Itaconic acid, a methylene succinic acid, has been discovered to involve in host-parasitic co-immunization through macrophage activation [31, 32]. 2-Hydroxyglutaric acid has been used as an endogenous MRI probe due to its well humoral adaptation and the great ability to precisely locate the tumor tissues [33].

2.3 Nucleosides and Nucleotides

Nucleosides and nucleotides not only participate in the macromolecular biosynthesis of DNA and RNA to assemble genetic materials, they are also direct energy substrates. It is well known that ATP is the basic energy source for many irreversible processes of biochemical metabolism and transmembrane transport of substances [34]; GTP-binding proteins constitute an important molecular switch on the cellular membrane systems, which regulates the substrate-level phosphorylation process of proteins [35]. Meanwhile, cyclic nucleosides are important second messengers in GPCR-mediated signaling pathways. cAMP can be involved in the glycogen metabolism and regulation of gene expression [36, 37], as well as regulating the activation and inhibition of many drug receptors like adrenergic receptors and M-acetylcholine receptors [38, 39]. The cGMP regulates the switch of the photo-controlled cation channel [40] to produce visual effect [41, 42]. GDP and GTP can also activate the tyrosinase-mediated RTK-Ras pathway by Ras receptors for gene expression regulation [43]. Nucleosides and numerous modified nucleosides, such as methylation modification [44–46], hydroxyl modification [47–50], aldehyde modification [51], and carboxylic acid modification [52], have important guiding significance for epigenetics of genes.

2.4 Sugar and Phosphate

Carbohydrates are one of the most complex polar molecules due to their complex stereoisomeric forms. Different bonding types lead to biomacromolecular polysaccharides with diverse structures and functions. The detection of monosaccharides and the differentiation of isomers are keys to elucidating the biological function of the composition of polysaccharides. Glucose metabolism is the basic energy metabolism of the organism, converting the energy substance glucose into adenosine triphosphate (ATP) that can be directly utilized. The sugar metabolism in cancer cells is distinguished from normal tissue due to the Warburg effects [53] referred above and is closely related to neurodegenerative diseases [54]. The pentose phosphate pathway not only produces glucose phosphate and fructose phosphate required for glycolysis but also provides reduced Nicotinamide Adenosine Dinucleotide Phosphate (NADPH). Notably, it was reported that sedoheptulose-7-phosphate is a potential marker for the evaluation of transaldolase deficiency [55]. Many sugars are only found in fungus or plants, which can be an evaluation index for dietary intervention and exposome research.

2.5 Water-Soluble Vitamins and Cofactors

Cofactors are usually the derivatives of vitamins which are famous for NAD $(P)^+$ and NAD(P)H. The redox states of NADH and NADPH are important criteria for the evaluation of cellular energy metabolism or oxidative stress [56, 57]. Since cofactors and vitamins are mostly involved in the hydrogen transfer redox process without the formation of carbon skeletons, their physiological functions were overlooked in the past decades [58]. However, studies have shown that the reconstitution of nicotinamide is associated with the process of deacetylation of histone, which can prolong the lifespan of mammals through increasing the activity of NAD synthase [59–62]. NADP (Niacin adenosine dinucleotide phosphate) can mediate the calcium trigger-calcium release mechanisms without passing through the calcium reservoir in the endoplasmic reticulum or sarcoplasmic reticulum, promoting muscle contraction, catecholamine secretion, insulin secretion, and T cell activation [63–65]. Folate metabolism provides a source of methyl for cells and has important regulatory power for DNA methylation [66]. Recent research confirms that vitamin C can participate in a new modification of DNA called glycerylation [67].

2.6 Carnitine and Choline Metabolites

Carnitines are quaternary ammonium carboxylate derivatives binding to fatty chains to assist in fatty acid transmembrane transport for β-oxidation [68], which is reported to have a close relationship with inborn errors metabolism [69, 70], type II diabetes [71, 72] and tubular nephropathy [73, 74]. Choline is involved in the formation of lecithin in animal tissues, and as a precursor, it synthesizes acetylcholine (ACh) and participates in neuromodulation. Betaine acts as a methyl donor, activates AMPK, and regulates cell osmotic balance [75].

3 Challenge of Analysis for Hydrophilic Metabolites

3.1 Influence of High Hydrophilicity on the Detection of Chromatographic Mass Spectrometry

Gas chromatography–mass spectrometry is widely used in the detection of amino acids and organic acids in the early years due to its abundant cleavage information and database [76–78]. However, GC–MS analysis often needs complex derivatization procedures and is not suitable for high-boiling point substances and unstable compounds like nucleotides and keto acids, which hampers its applications in the analysis of a wider range of hydrophilic metabolites. Traditional reversed-phase liquid chromatography (RPLC) uses a hydrophobic C18-terminal non-polar stationary phase, hence it is difficult to form a strong hydrophobic interaction with hydrophilic metabolites, therefore, cannot retain hydrophilic metabolites on the column. The development of new stationary phase and mobile phase additives to enhance the hydrophilic interactions have become important strategies for the analysis of these substances.

3.2 High Dynamic Range

Human Metabolome Database Bank (HMDB) lists the distribution of concentration of hydrophilic metabolites in normal serum samples [6]. The summary of selective metabolites is shown in Fig. 1. The wider dynamic range has been illustrated; for example, the content of hormones is at the picomole range, amino acids are often maintained at micromole levels, some organic acids and sugars can go up to millimole levels in the plasma samples. The levels of lactic acid are typically related to the metabolic status. In normal conditions, it is below 4 mM; however, when subjected to intensive excise, it could even reach to 18 mM [79, 80]. In urine, there is also a wide concentration range of metabolites given the wide range of dilution factor in urine samples. A high abundance of hydrophilic metabolites produces strong ion suppression in mass spectrometry detection, which will affect the ionization and detection of low abundance metabolites. At the same time, a large number of similar structures that tend to elute at the same time will lead to cross-interfere in mass spectrometric channels. This creates great challenges for quantitation.

3.3 Stability

Although hydrophilic metabolites have a small molecular weight and a simple structure, there are still a large number of easily decomposable and transformable species. Keto acids are unstable to both heat and light but play an important role in the Krebs cycle. In particular, oxaloacetic acid and α-ketoglutaric acid are easily decarboxylated in sample pre-processing; pyruvic acid is a light-sensitive carboxylic acid, which undergoes self-isomerization and redox reaction under light [81]. Nucleoside triphosphates may undergo a reversible phosphate exchange reaction ($\Delta G = 0$) between NTP and NDP under the catalysis of nucleotide kinase and Mg^{2+}, which leads a problem to quantify the NTPs and NDPs accurately. For example, the ATP hydrolysis [82, 83] is a spontaneous process ($\Delta G \sim -30.5$ kJ mol^{-1}) so that ATP often undergo obviously in-source dissociated [84]. $NAD(P)^+$ and NAD(P)H are the electron carrier with very strong redox capacity (the standard oxidation–reduction potential $E^{0-} = -0.32$ V), which leads to the uncertainty for quantification of NADH when samples expose to air. SCFs are always lost during extraction at room temperature due to high-saturated vapor pressures and low boiling points. For example, the boiling point of butyric acid is at 88 °C and the vapor pressure can reach 0.1 kPa. Coenzyme A is also unstable to the heat. Care should be taken when quantifying these classes of unstable metabolites.

4 Applications of Chromatographic–Mass Spectrometry in Detection of Hydrophilic Metabolites

4.1 Gas Chromatography–Mass Spectrometry (GC–MS)

As mentioned above, GC–MS can only detect thermally stable and easily gasified metabolites; therefore, GC–MS is suitable for the detection of fatty acids, amino acids and small organic acids. In view of the strong polarity and high boiling points of amino acids and organic acids, derivatization by converting them to the respective esters has become mostly used methods. Derivatization methods for GC–MS mainly include silanization and esterification. Silanization used N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA),

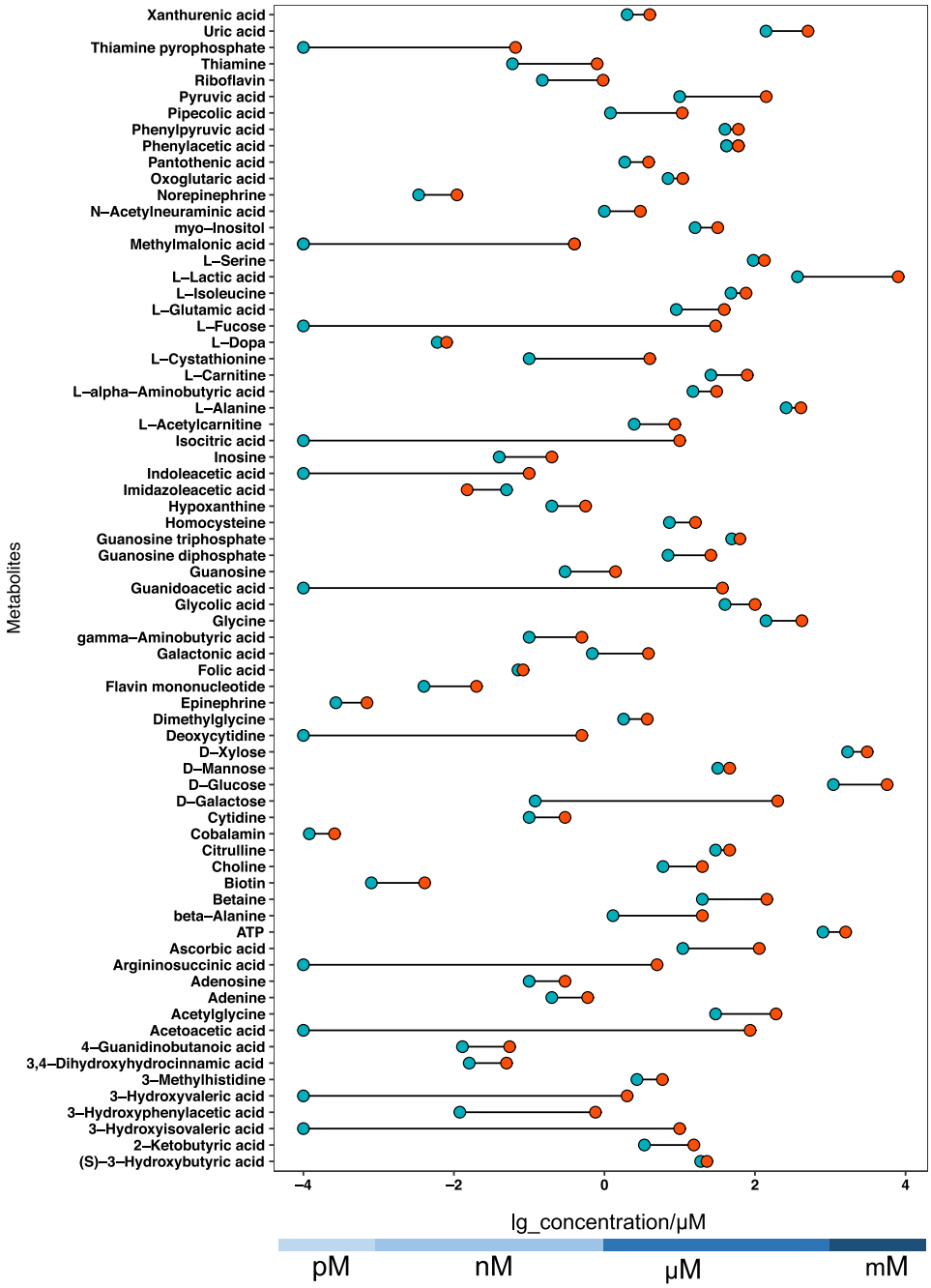
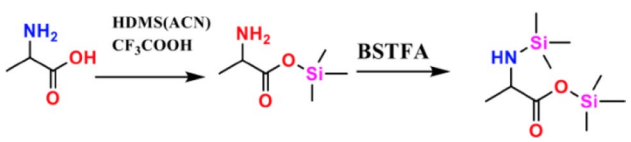


Fig. 1 Concentration range of partial hydrophilic metabolites in human serum and urine Data source is from HMDB and the figure is drawn by R 3.5.3 with ggplot2 package

hexamethyldisilazane (HMDS) [85] or trimethylchlorosilane (TMCS); these compounds can react with hydroxyl and amino groups simultaneously. The reaction principle is shown in Scheme 1. The reaction requires organic solvent systems, which limits its applications for detection of water-soluble hydrophilic metabolites in biological samples. In addition, it is not recommended to apply this method for the detection of sugars due to the complexity of the fragmentation of silanized products produced [86]. Acetyl chloride [87–89] or other active esters [90] have strong reactivity with volatile alcohols or fatty acids at low temperatures, so they



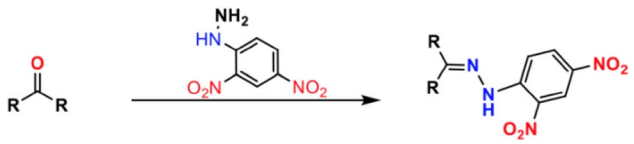
Scheme 1. Derivatization of alanine with BSTFA and HMDS in GC–MS analysis

are often used for the detection of organic acids. The detection of aldehydes, ketones and sugars often uses 2,4-dinitrophenylhydrazine [91, 92] to transform the carbonyl to oxime. The reaction principle is illustrated in Scheme 2.

Notably, most nonvolatile metabolites cannot be detected by GC–MS even after derivatization because the products still have a much higher boiling point. In addition, multi-origination and multi-peak phenomena due to interferences between metabolites of interest and other metabolites containing similar structural units hamper wider applications of GC–MS. One of the examples is the measurement of fatty acid, which could have interference from the fatty acid unit of phospholipids [93].

4.2 Reversed-Phase Liquid Chromatography–MS (RPLC–MS)

RPLC–MS is a classic separation method in MS-based metabolomics research, including untargeted profiling and targeted quantification [94, 95]. Due to the hydrophobic nature of C18 columns, they cannot retain hydrophilic metabolites, such as amino acids and the metabolites in TCA cycles like citric acid. T3 column is a relatively better choice for retention of polar metabolites as the column reserves parts of silicon hydroxyl in the particle's surfaces compared to C18 column. Xu's group developed the T3 methods in 2D-LC system to achieve the detection of both hydrophilic and hydrophobic metabolites at the same time. They use T3 and BEH C18 column to cover the short-chain and long-chain acyl Coenzyme A esters [96], which increased the detection efficiency and throughput. However, the hydrophilic interaction with the T3 column is still not strong enough for retaining the small molecular metabolites.



Scheme 2. Derivatization of carbonyl group with DNPH in GC–MS analysis

4.3 Hydrophilic Interaction Liquid Chromatography–MS (HILIC–MS)

HILIC is firstly proposed by Andrew Alpert [97] in 1990. He used a polar stationary phase and an organic solvent containing only 2–3% water as the mobile phase in the RPLC system to achieve the analysis of hydrophilic metabolites. The stationary phase used in the HILIC column is generally a polar end-capped silica gel, including a non-modified silica gel [98] and a modified silica gel with functional groups of amide (Amide), aminopropyl, diol, and zwitterionic, etc. [99, 100]. (Fig. 2a). The well-recognized HILIC retention principle is shown in Fig. 2b, the polar terminal rapidly forms a water layer on the surface of the stationary phase in the initial high percentage of organic phase. The efficiency of separation depends on the distributions of analytes in water layer and organic layer [101, 102].

HILIC columns provide a new strategy for the separation of amino acids, organic acids, carbohydrates, choline, carnitine, neurotransmitters, nucleosides and nucleotides in the hydrophilic metabolites, some recent advances have been listed in Table 1. Recently study suggests that zwitterionic sulfobetaine ZIC-HILIC is more suitable for global metabolic profiling compared to an underivatized silica HILIC stationary phase in terms of chromatographic peak shape and resolution as well as metabolite coverage [103]. Although HILIC can enhance the retention ability of these metabolites, low sensitivity and poor peak shapes remain to be problems for organic acids and phosphorylated metabolites. Recently a new solvent additive, medronic acid has been developed to reduce the chelation between metabolites

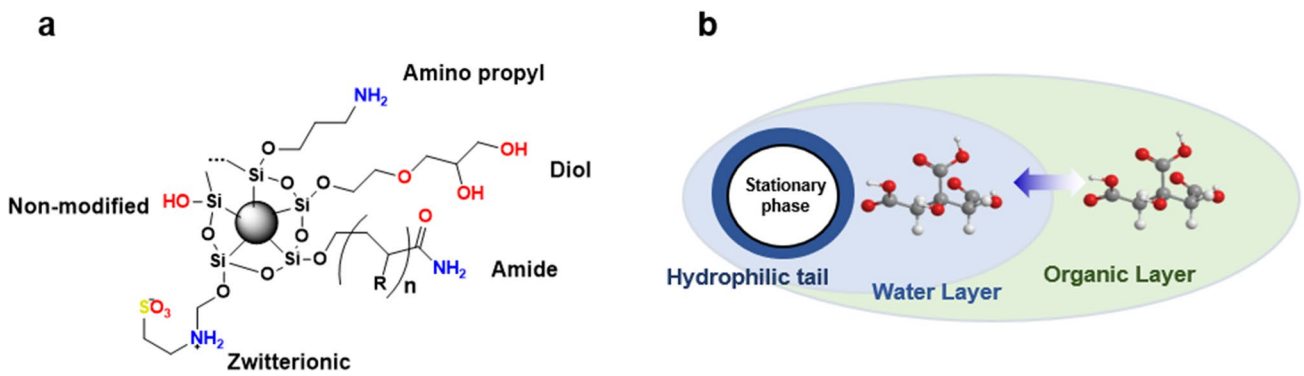


Fig. 2 The stationary and the mechanism of HILIC. **a** The packing materials of the stationary phase commonly used for HILIC analysis; **b** schematic diagram of the retention mechanism. This figure is drawn by ChemBioDraw 2012 software and Microsoft PowerPoint

Table 1 Partial applications using HILIC to achieve analysis of polar metabolomics in recent years

Analytes or objective	Year	Column	MS instrument	Highlights	References
Amino metabolites	2019	ACQUITY BEH Amide column	QQQ	The LOD for transmitters is below 10 ng/mL, and most metabolites have quantitative range from 25 to 20,000 ng/mL The LOQ for 40 amino acids was in the range of 0.6–10 ng/mL	[105–107]
Acylcarnitine	2017	Syncronis HILIC column Kinetex HILIC silica column	Orbitrap QTrap	Both quantification and identification ability to analyze carnitines without standards via High resolution mass spectrometer A more simple and precise quantification method via LC-MRM	[108, 109]
Phosphocholines	2016	Phenomenex Luna HILIC column	QTrap	Applicable for EDTA-plasma, serum and urine samples with high accuracy and precision	[110]
Amino acids	2016, 2017	ACQUITY BEH Amide column	QQQ	13C-Glutamine as a tracer to monitor the change of amino acids in cells The isomeric amino acids such as Leu, Ile and allo-Ile can be separated	[111, 112]
Phosphorylated surgas	2016.2017	ACQUITY UPLC BEH Amide	Q-TOF	Methylphosphonic acid can improve the separation of phosphorylated sugars Trehalose-6-phosphate quantified first time in tissues of legume *M. truncatula*	[113, 114]
Nucleotides	2020	ACQUITY BEH amide column	QTrap	LOD: nucleoside triphosphates: 5000–10,000 ng/mL, nucleoside diphosphate: 1000 ng/mL, nucleoside monophosphate: 50 ng/mL	[115]
Methylated metabolites	2014,2019	Hypersil GOLD aQ column XBridge BEH Amide column Luna-NH_2 column	QQQ	The first systematic research of analysis of methylated nucleosides in t-RNA Apart from nucleosides, methylated amino acids, organic acids were also considered of 20 methionine related methylated metabolites were revealed	[116–118]
Phosphorylated and carboxylated metabolites	2018	Poroshell 120 HILIC-Z column	Q-TOF	Medronic acid as a new solvent additive can improve the signal and peak shapes which effected by metal-chelated	[104]
TCA metabolites	2019	ACQUITY BEH amide column	QQQ Q-TOF	Both QQQ and QTOF method to realize ^{13}C metabolic flux analysis A systematic protocol for performing HILIC assisted ^{13}C flux research Network-wide metabolic pathway elucidation assisted by ^{13}C tracer	[119–120]
Sulfur pathway metabolites	2015	Silica-based NUCLEODUR HILIC column	TOF,QQQ	Thiol derivatization assisted HILIC- and RP-LC	[122]

and metal ions in 2018 [104]. Unlike traditional ion-pairing agents with ion suppression, medronic acid can ameliorate containment in LC system.

HILIC coupled with MS has been incorporated in two dimensional (2D) LC and multi-omics acquisition platforms. HILIC has become a great method when combined

with RPLC to simultaneously cover the analysis of polar and nonpolar metabolites. A parallel column regeneration with 2D LC method has been implemented to increase the throughput of measurement whilst achieving high coverage of metabolites [123] (Fig. 3). Whilst the first set of 2D columns are accountable for separating analytes, the second set of 2D columns are conditioning, which is essential for achieving data consistency with high efficiency. Recently, a RPLC–HILIC-tailored SRM strategy has been proposed for simultaneously detecting large-scale targeted metabolomics and proteomics, where about 101 metabolites and digested peptides have been detected, demonstrating the benefit of the integrated chromatographic spectrum [124]. Nevertheless, due to the complexity of interactions in HILIC, the stability of chromatographic retention time and the peak broadening are remained to be the problems for the HILIC method, hence they are still in need of further improvement [125].

4.4 Capillary Electrophoresis–MS (CE–MS)

The electrophoresis separation is driven by electrodynamics and charged ions are separated according to their own mobility under an electric field maintained by a certain voltage [126, 127]. The main component of the capillary tube wall of the stationary phase is the silanol group that is formed with SiO^- group to attract positive ions under acidic conditions, and generates electro-osmotic flow (EOF) at the interfacial electric double layer spontaneously, as shown in Fig. 4. When the pH of the buffer is above 4 (p*K*a of silanol group), the zeta potential would increase and reach the maximum at pH ~ 7 as a consequence of the increase of the charge density on capillary surface. The drawback of this method is poor reproducibility due to the instability of EOF resulted from the changes of the electrochemical properties of silica surface (Table 2).

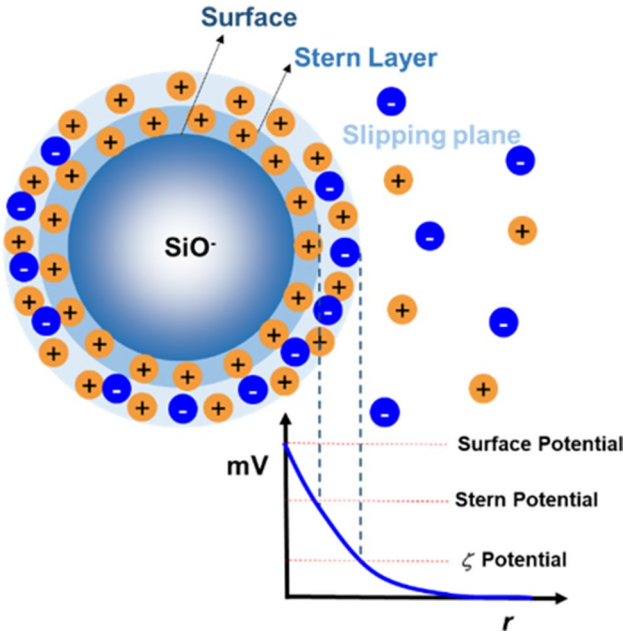


Fig. 4 Electric double-layer model and Zeta potential, which was drawn by Microsoft PowerPoint

The approach based on capillary electrophoresis–mass spectrometry is a suitable for polar metabolomics analysis, especially cation and anion metabolites. Soga et al. [128] firstly used the CE–MS method to measure plasma metabolite profiles in 2003 and performed a cohort analysis of 8000 people, including both anionic metabolites such as small molecular carboxylic acids like lactic acid, malonic acid, citric acid and cationic metabolites such as amino acids, creatine, betaine, etc. This work provided an important reference for the determination of the absolute concentration of hydrophilic metabolites in plasma.

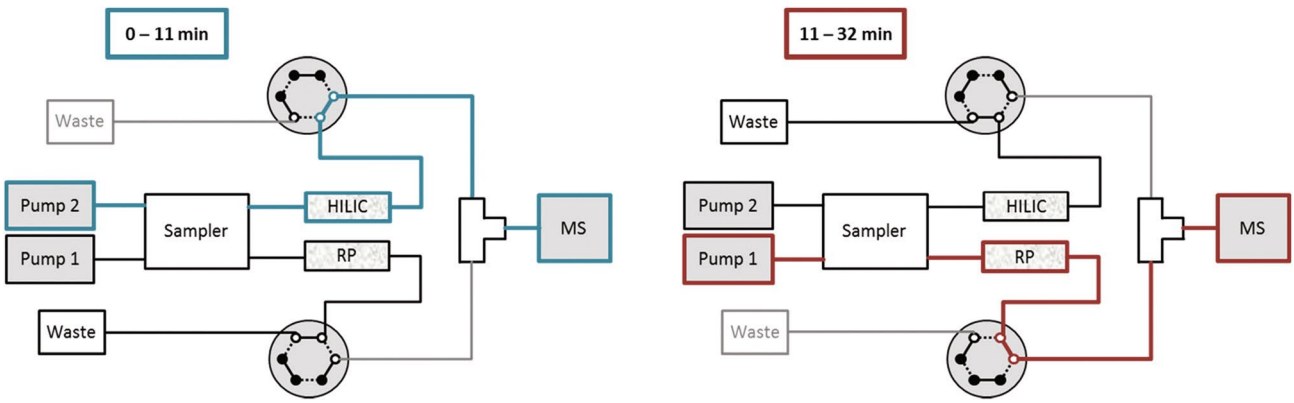


Fig. 3 The parallel column regeneration method for analysis of metabolites and lipids consecutively. The blue line and red line represent the two independent flow-paths. Among them, the blue line with 11 min is HILIC elution of hydrophilic metabolites to MS, followed by RP elution of lipids in the red line. During the running of each column, the other column undergoes re-equilibration to a waste bottle. Reprinted with permission from [123]. Published by The Royal Society of Chemistry (RSC)

Table 2 Partial applications used CE–MS to achieve the analysis of polar metabolomics in recent 5 years

Samples	Year	Background electrolyte/running voltage	Analytes	Highlights	References
Blood	2018	1 M formic acid/30 kV	54 cations, 40 anions	64 metabolites(CV < 20%) can be detected, the CE-MS is suitable for large-scale epidemiological studies for its stability	[128]
Blood	2015	1 M formic acid/30 kV for cation metabolites 50 mM ammonium acetate/− 30 kV for anion metabolites	70 polar metabolites	70 metabolites covered glycolysis, TCA cycle, nucleotide synthesis were detected. Sample incubation time, temperature, and freeze–thaw cycle were optimized	[129]
Muscle tissues	2019	1 M formic acid with 15% acetonitrile (pH 1.8)/30 kV for cation metabolites, 50 mM bicarbonate(pH 8.5) for anion metabolites	80 polar metabolites	A new approach called multisegment injection-CEMS (MSI–CE–MS). Sample extraction method development with only 5 mg freeze-dried tissue	[130]
Cells and tissues	2018	5 mM ammonium formate 10% methanol (pH 2.5)/20 kV	15 metabolites	Amines, hydroxyls and carboxylates were derivatized with tertiary amines tags	[131]
Blood Spots	2019	1 M formic acid with 15% acetonitrile (pH 1.8)/30 kV	Untargeted profiling	The first nontargeted metabolomics study for cystic fibrosis in dried blood spots	[132]
Urine	2018	0.1 M formic acid (pH 2.4)/20 kV	TMAO, carnitine, creatine	The dual detection coupled with UV and MS to solve the problem of different concentration of metabolites	[133]
Cerebrospinal fluid	2018	150 mM acetate (pH 3.7, adjusted by NH_4OH)/30 kV	Amino acids	Chiral separation of amino acids with derivatization of FLEC The LOD of amino acids are almost below 0.2 μM	[134]
Embryo	2018	1% formic acid/20–22 kV for cation metabolites 20 mM ammonium bicarbonate/17–19 kV for anion metabolites	~60 cation metabolites ~24 anion metabolites	Single-cell CE-MS detection The LOD is below 5–10 nM	[135]
Cell	2019	1 M formic acid in 10% methanol /30 kV	57 polar metabolites	LC–MS, GC–MS and CE–MS profiling were compared in this article	[136]
Baby food	2017	1.6% hexafluoro-2-propanol	Nucleotides	The LOD of nucleotide mono-,di-and tri-phosphate are 7–23, 20–49 and 64–124 ng/mL respectively	[137]

The most significant advantage for CE–MS is its considerable success for profiling extremely small amount of samples [138, 139]. It is an essential methodology to achieve single-cell metabolic flux analysis. In addition, CE–MS exhibits the superiority of stereoselective separation for chiral metabolites [140, 141]. Furthermore, as an important tool for single-cell detection, CE–MS also performs well in the field of in situ analysis. A special CE–MS device without sampling extraction has been developed [142], which can detect a total of 13,000 molecule features with precise the localization in various rat tissues. Notably, the ability to separated isobaric and isomeric metabolites, including valine and betaine improves the confidence for identification. The in situ sampling mass spectrometry coupled with CE separation could be the next generation of mass spectrometry imaging (MSI) as it adds another dimension compared to traditional MSI and could also have the potential for single-cell imaging analysis.

Due to a lack of pressure-driven laminar flow, eddy current diffusion and mass transfer resistance, CE is generally more efficient than HPLC. However, it is also worth noting that when a low amount of sample performed, the sensitivity would be lower due to the post-capillary dilution effect produced by the axillary interface [143]. The separation efficiency of CE is closely related to the electric field strength but Joule heat and electrodynamic dispersion can cause peak broadening [144]. The use of a commercial 30 kV voltage and even the currently reported 120 kV instrument requires a good electromagnetic shielding and heat sink [145]. CE also requires a strong acid–base balance system before and after MS analysis [141].

The development of CE instruments has greatly improved the sensitivity in metabolomics. To address the effect of the axillary interface on sensitivity, Tseng et al. [146] have developed a beveled tip sheath interface that could enhance the detection sensitivity. Hirayama et al. [147] developed a sheathless interface coupled with the mass spectrometer, achieved the analysis of 52 cationic metabolites with only 1.4 nL injections, including amino acids, nucleobases, nucleotides, etc. and the LOD values of those ranged from 30 to 1000 nM.

Most CE approaches need two buffer components to detect both anionic and cationic metabolites, and the lower flow rate leads to a long time to accomplish full analysis. Additionally, the lack of reliable repeatability [128, 148] also limits its application. Drouin et al. [149] used a two-step CE-MS that overcomes the peak broadening caused by the suction effect of the nebulizer. This new CE–MS enables simultaneous analysis of anion and cation metabolites in the same buffer system. In summary, CE–MS has the potential capability in single-cell hydrophilic metabolomics and in situ localized analysis. Analysis efficiency still needs further improvement.

4.5 Ion Chromatography–MS (IC–MS)

Ion chromatography also called as ion-exchange chromatography, is well applied in the ionic compound analysis [150]. According to the charge and size of analyte ions, the oppositely charged stationary phase is used to build electrostatic interactions. In the past, IC is not suitable for couples with MS detection, because the strong electrodes in the mobile phase are not friendly to MS detection. The development of ion suppression technology for elute, especially continuous on-line desalination, has revolutionized the filed, which allows IC and MS to be compatible. The suppressor is always composed of ion-exchange resins or electrochemical films which transforms strong background electrolytes to volatile weak electrolytes (e.g. Thermo Scientific Dionex AERS500 anion electrolytic suppressor can convert the potassium hydroxide gradient into pure water [151]), which can overcome the issues, such as environmental pollution, stability, and reproducibility. However, many hydrophilic metabolites with weak acidity are also being suppressed at the same time, leading to poor sensitivity. In recent years, attention has been paid to IC–MS research due to its favorable capability of separation hydrophilic metabolites and even strong polar ions, ion suppression issue has been extenuated by adding low concentrations of volatile acids or bases to enhance MS signals [152].

The IC–MS has been applied to targeted metabolic profiling and has demonstrated its huge potential for analysis of polar metabolites, especially polycarboxylic acids and nucleotides [153]. The limits of quantification in this study are from 0.25 to 50 μM with the interday assay precisions ranged from 1 to 19%. Detection of these metabolites using HILIC–MS has been proven unsatisfactory. Moreover, isomeric metabolites, such as citric acid and isocitric acid, show great separation in base conditions with IC–MS system which could not be obtained by HILIC or Ion-pairing RPLC methods. Although IC–MS can achieve favorable separation, reproducibility and sensitivity for polar metabolites, the efficiency of IC–MS approach raises certain concerns as it normally takes 20 min to complete one run of analysis. We suspect that by adding ammonium in the regeneration step could enhance the MS intensity in the negative mode as opposed to the methanol-pure water used in the study.

With the development of the capillary ion exchange column, the sensitivity for IC–MS to detect hydrophilic metabolites has increased. Figure 5a shows the equipment of an CapIC–MS. Due to its improved selectivity, IC–MS has achieved preliminary applications in sugar, organic acid, phosphate and nucleotide analysis [154–157].

Huang et al. used a commercial CapIC-Q/Extractive MS system to perform anion-untargeted metabolic profiling [157] and targeted quantitative analysis of head and neck squamous cell carcinoma cells (HNSCC) [151]. Compared with

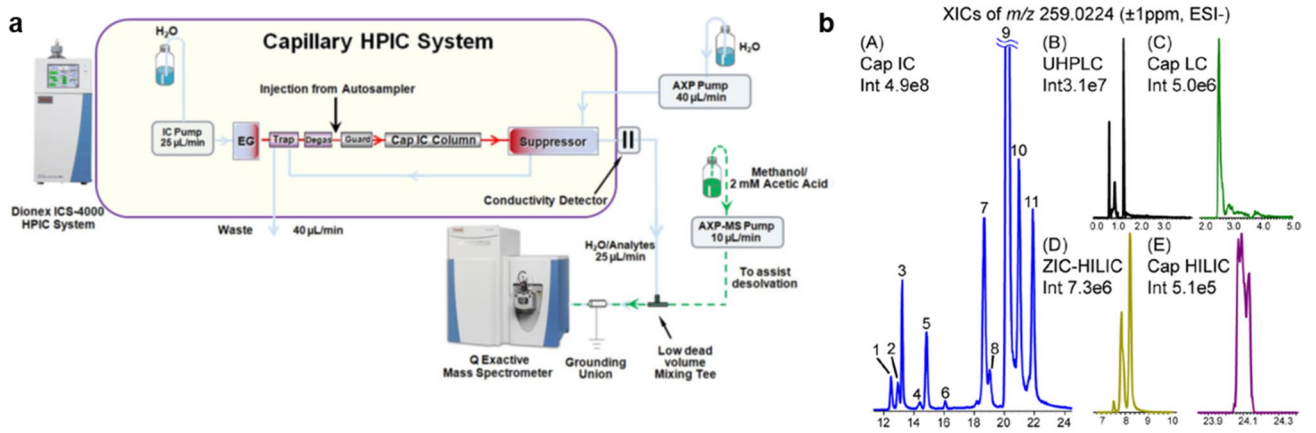


Fig. 5 The IC-MS for analysis of hydrophilic metabolites. **a** Thermo CapIC-Orbitrap Q/Extractive MS structure. Reprinted with permission from [157]. **b** CapIC/HILIC/RPLC–MS extracted ion map of hexose phosphate in UM1 oral cancer cells. The explanation of figure number in original figure is: **a** Cap IC, **b** UHPLC, **c** Cap-LC, **d** ZICpHILIC, and **e** Cap-HILIC. Reprinted with permission from [157]. Copyright 2014 American Chemical Society (ACS)

RPLC and HILIC, CapIC–MS has a nearly 100-fold increase in detection sensitivity with a LOD value of 0.2–3.4 fmol. Figure 5b compares the sensitivity of the ion chromatograms of CapIC, HILIC and UHPLC when testing of 1 ppm hexose phosphates. It can be seen that CapIC exhibited not only high sensitivity, but also good separation for isomers. In terms of quantification, CapIC/Q Extractive HF can accommodate a wide dynamic range (5 orders of magnitude) and quantify accurately Krebs cycle metabolites ($R^2 \sim 0.99$), achieving full coverage detection of metabolites from fmol/L to nmol/L.

Sun et al. performed serum and urine metabolic profiling via CapIC–MS and a total of 131 polar metabolites have been identified and quantified. This includes metabolites in TCA cycle, gloxylate and dicarboxylate metabolism, amino acid metabolism and pentose phosphate metabolism [158]. CapIC–MS has also found applications in areas of cellular immunology [159] and the functional of metabolomics [160].

Although IC–MS has played an important role in hydrophilic metabolites analysis, the runtime is about 60 min, which limits its application in cohort studies with large amounts of samples. In addition, realizing high coverage of both cationic metabolites and anionic metabolites needs two ion exchange columns and elute systems, which escalates the issue of low-throughput for clinical applications. As for an important approach for the simultaneous analysis of both polar metabolites and inorganic ions, improvement is necessary to meet the demand.

4.6 Ion Pairing Reversed-Phase Chromatography–Mass Spectrometry (Ion Pairing, IPRPLC–MS)

The IPRPLC–MS can enhance the hydrophobic and electrostatic interactions between the polar analytes and the stationary phase by adding ion-pairing reagents (IPR) to obtain better retention behavior. The use of a C18-terminal as stationary phase with ion-pairing reagents could greatly improves the stability and reproducibility of metabolic detection compared to the use of HILIC.

The IPRs are normally volatile amines and analytes using IPRs as mobile phase are often detected with negative mode. The common IPRs are triethylamine (TEA) [161, 162], tributylamine (TBA) [163–166], diisopropylethylamine (DIPEA) [167, 168], hexylamine(HXA) [169–171], etc. They are added in cooperation with charge balance reagents such as HAc [171] and hexafluoroisopropanol (HFIP) [162, 167, 168]. The hydrophobic carbon chain of the amines would form a hydrophobic interaction with the stationary phase, and the amino group after ionization would form an electrostatic interaction with the analytes. Hence, the ion exchange principle can be realized in a RPLC system.

The retention mechanism of IPLC is still controversial. It is mainly divided into two stoichiometric mechanisms [172–174] based on ion-pair model and dynamic ion-exchange model, and a non-stoichiometric model based on the electric double-layer model [175]. As shown in Fig. 6a, the ion pair model [173] considers that the IPR and the analytes in the solution are firstly combined to be neutral ionic molecule and then form a hydrophobic interaction with the stationary phase. The dynamic ion-exchange model [176] put forward a theory that the aliphatic chain of IPR firstly non-covalently bounded to the stationary phase, and then the head group of IPR with polar groups would interact with metabolite with opposite charges. Both of the models lack experimental evidence, nevertheless, they can provide a thermodynamic equation to describe the retention index. However, Knox and Hartwick et al. argued that these two models have the same initial and final states, which caused

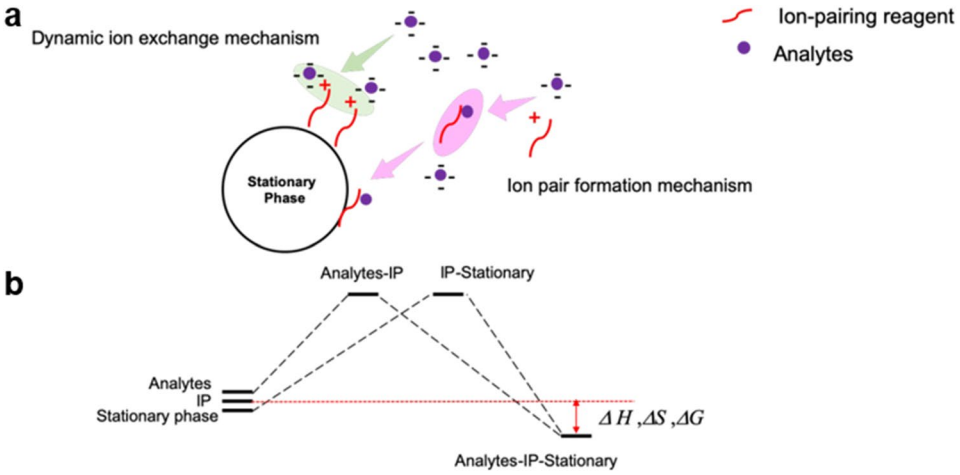


Fig. 6 Ion pairing chromatography mechanism. **a** The dynamic ion-exchange process is the green arrows part; the ion-pairing mechanism is the pink arrows part. **b** The thermodynamic processes of these two mechanisms. This figure is drawn by Microsoft PowerPoint

their thermodynamically indistinguishable (Fig. 6b). The electric double layer model proposed by *Bidlingmeyer* is based on experimental facts [174]. It is believed that the non-covalent bonding of the IPR to the surface of the stationary phase produces an electric double layer, and the adsorption process of the analytes is modulated by the electrostatic potential. Although the electric double layer model considers the effects of electric field force, such as, van der Waals force, hydrophobic interaction and electrostatic interaction, for chromatographic retention, it is limited due to the inability of quantization. The relationship between reagent concentration and the stationary phase is still best descripted by the stoichiometric model for the prediction of the retention behavior [177].

Ion pairing chromatography owes an irreplaceable advantage in the analysis of hydrophilic metabolites, especially small molecular acids [170], nucleotides and coenzymes A [166, 178]. Notably, zwitterions or metabolites that are difficult to ionize, such as amino acids, sugars, etc., are not separated well in negative ion pair chromatography [178]. Gong et al. [162, 168] evaluated the separation of oligonucleotides using negative IPR additives like TEA, TBA, HXA, etc., and found the optimal concentration of IPR and HFIP. Although most IPLC mainly deals with anionic metabolites, there are a few studies developed positive IPLC methods. Li et al. [179] used hexafluoro butyric acid (HFBA) as a positive IPR to detect amino metabolites.

The biggest drawback of IPLC is the permanent contamination of LC–MS system [167, 179]. Increasing the carbon number of the amine could enhance the interaction with the stationary phase, but at the same time will increase their boiling point, which is not compatible with mass spectrometers. TEA and TBA have been shown to produce the non-removable signals [180] of $m/z = 102$ and $m/z = 186$, respectively. Guo et al. [167] found that DIEPA is more volatile with better compatibility with the mass spectrometer. Li et al. found that IPLC required a long equilibration time to achieve reproducibility and the use of 2D-LC switching could avoid the contamination because ion-pairing reagents could be eluted in the 2nd dimension of LC void [179]. Table 3 shows the results of nucleotides and polysaccharides analyzed with TEA, TBA, HXA as IPR. The ACQUITY UPLC BEH C18 column is most widely used in IPLC system due to its good tolerance of pH range (1–14) and temperature. In addition, hexafluoroisopropanol (HFIP) has high vapor pressure and volatility, which can enhance

Table 3 Metabolome analysis of TEA, TBA, HXA as ion pair additives

Analytes	Column	Ion-pairing reagents	References
Nucleotides, coenzyme A	ODS-3 cartridge column	5 mM HXA + 10 mM NH_4Ac 10 mM TBA + 15 mM NH_4Ac	[165, 178]
Oligosaccharides	ACQUITY UPLC BEH C18	5 mM HXA + 100 mM HFIP	[181, 182]
Glycosaminoglycans	ACQUITY UPLC BEH C18	5 mM HXA + 100 mM HFIP	[183–185]
Oligonucleotides	ACQUITY UPLC BEH C18	5 mM HXA + 100 mM HFIP	[162, 168, 186]
Small molecule organic acids	ACQUITY UPLC BEH C18	5 mM HXA + 10 mM NH_4Ac	[165, 170]
Sugar phosphate	Luna C18 column ACQUITY UPLC BEH C18	5 mM HXA + 10 mM NH_4Ac	[165, 171]
Cofactors (NADH, etc.)	ACQUITY UPLC BEH C18/T3 Luna NH_2 column	10 mM TBA + 15 mM HAc 20 mM TEA + 20 mM NH_4Ac	[163, 187, 188]

the ionization of negative ion metabolites in the ESI source to improve the analytical sensitivity [167]. The development of IPR without containment will be revolutionary in the IPLC methods.

5 Chemical Derivative Assisted Hydrophilic Metabolites Analysis

The RPLC-MS has better repeatability, robustness, and long lifetime of columns owing to the nature of the packing material of the reversed-phase column. However, the interaction between the hydrophilic metabolites and the C18-terminal phase is too weak so that they tend to flow out in the chromatographic dead volume. Despite the use of the new separation technique described above, the problem of low sensitivity of acidic metabolites in the negative ion mode of mass spectrometry remains unresolved. The development of chemical probes with enhanced chromatographic retention and the mass spectrometric response has become a solution for polar metabolite analysis.

An excellent probe should consist of four parts [189]: (1) a chemical reaction group can specifically react with a class of metabolites. Generally, the reaction for metabolites derivatization requires mild conditions, high reaction rate, and yield. Click reactions can meet these criteria and are often widely used [190–193]. (2) A hydrophobic group to enhance chromatographic retention: aromatic rings are preferred to be used for providing hydrophobic interactions, π–π interactions. (3) MS sensitivity-enhanced groups, especially strong bases with highly proton-capable ability, such as amino group, quaternary ammonium group. (4) Isotope reporting group, which can provide heavy isotope as an internal standard for every derivatized metabolite by chemical labeling. Meanwhile, the derivatized metabolites often have the same fragment ion produced by reagent via collision-induced dissociation (CID) so that it can provide rich information for quantification. Figure 7 shows the structure of derivatization reagent for amino acids [194]. According to the functional groups in metabolites, probes with specificity can be designed to greatly enrich the content of targeted metabolome analysis.

5.1 Derivatization of Amino Metabolites

Aliphatic amino acids are extremely difficult to retain on RPLC, and even using the IPLC, the retention is still weak due to their zwitterionic ionization and broad range of isoelectric points. It is worth considering that separating amino acids with different configurations of D- and L-types is also very challenging. A number of derivatization strategies have been developed for the analysis of amino acids, as shown by Scheme 3. Zhou et al. [195] used sulfonyl chloride to achieve chemical isotope labeling (CIL) for amino metabolites; Zhang et al. [196] utilized 6-aminoquinoline-*N*-hydroxysuccinimide ester (6-AQC) to increase the detection sensitivity of amino metabolites by 50–1000 times. It is obvious that the separation of natural amino acids with HILIC methods needs pH control whereas, with AQC derivatization, the amino acids are not only better retained on column but also increased MS response significantly. Wang et al. [197] modified the AQC method by replacing the quinolone ring with isoquinoline ring (5-AIQC), which has higher p*K*a value and is capable of analyzing more than 120 amino metabolites. This method enhanced sensitivity nearly 10 times compared to AQC. Derivatization method could also provide a solution for separation and quantification of the D-/L-configuration of amino acids in peptides via a chiral benzaldehyde probe as has been demonstrated by Pan et al. [194]. The principle is based on the stereoselective reaction between different substrates and reagents (like RR-, SS- and RS-, etc.) having different thermodynamic energy.

The nucleobases are also considered as amino metabolites because of their heterocycle containing nitrogen.

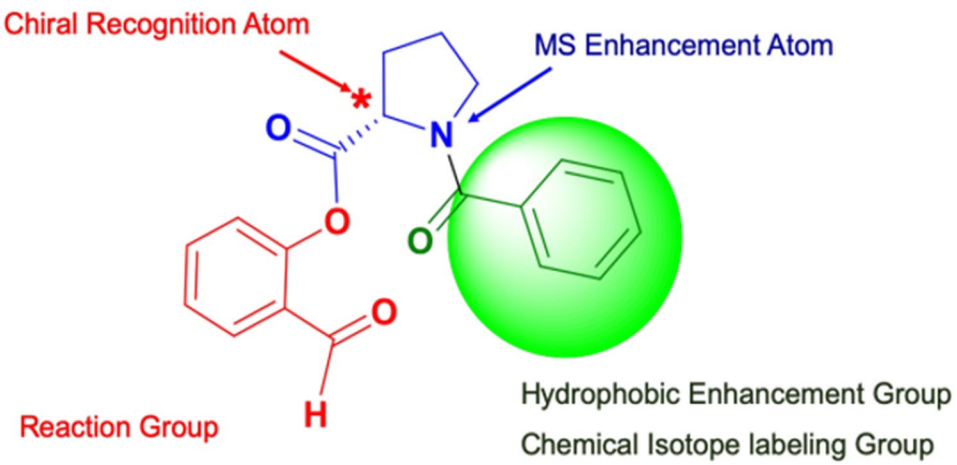
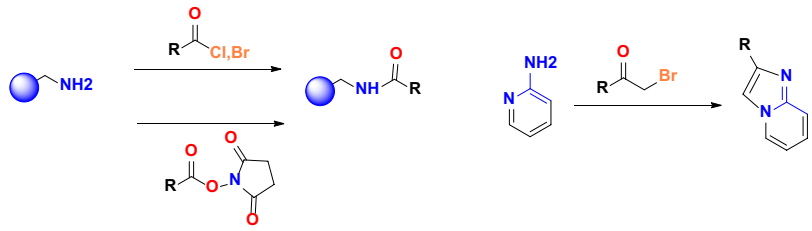
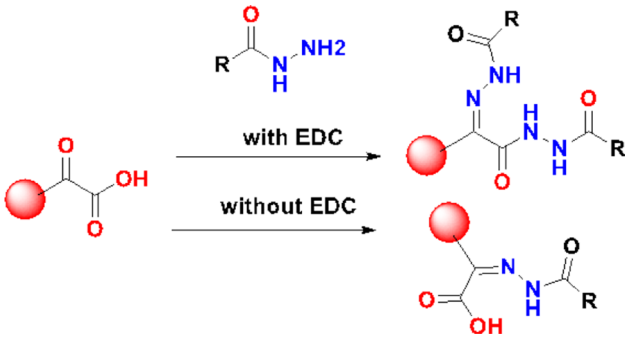


Fig. 7 Structural design of an amino acid derivatization reagent. (Ref. [194]), which is drawn by ChemBioDraw 2012

Scheme 3. Derivatization of amino group in LC–MS analysis





Scheme 4. Derivatization of Carboxyl group in LC–MS analysis

Quantification of nucleosides, especially methyl-modified nucleosides, is of greater interest to epigenetic researchers. Huang et al. [198] used an acyl bromide as a derivatization reagent to react with the amino group and their ortho-imine carried on the ring of anthracene or pyrimidine. They successfully quantified 5-methylcytidine (5-mC), 5-hydroxymethylcytidine (5-hmC), 5-aldehyde cytidine (5-foC), 5-carboxycytidine (5-caC) based on this approach.

5.2 Derivatization of Carboxyl and Phosphorylated Metabolites

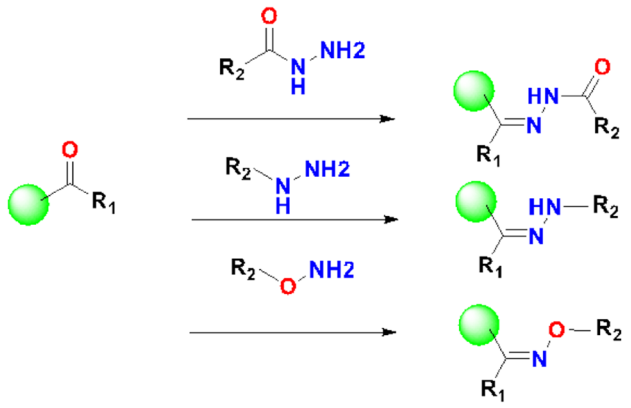
LC–MS analysis of phosphoric and carboxylic metabolites is even more challenging. This is because in addition to the poor hydrophobicity, these metabolites carry negative charges and the sensitivity of negative ion detection mode in mass spectrometry is lower.

Therefore, designing a suitable probe to sensitize these metabolites is more desirable. Derivatization strategies for carboxyl metabolites are shown in Scheme 4. Mark et al. [199] used EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) to activate the carboxyl groups and then transfer them into quaternary ammonium salt by 4-APEBA, which greatly enhances the sensitivity and hydrophobicity. Tan et al. [200] used EDC activation and derivatization with benzyl hydroxylamine, the products of which have the same reporter ion $m/z = 91$ and the LOQ was 0.3–300 pg. Li group [201, 202] and Jiang et al. [203] derivatized fatty acids with dansylhydrazine and dansylpiperazine, respectively, achieved LOQs ranging from 4–20 nM. In view of the instability of keto acids, Michael et al. [204] derivatized carbonyl groups with phenylhydrazine, then analyzed them with IPRPLC–MS method. This strategy uses the IPLC to achieve the retention of small molecular acids and increases the stability of keto acids at the same time. The sensitivity via transforming anionic organic acids to cationic derivatives to detect in positive ion mode is increased at least 100-fold.

Zeng et al. [205] achieved the detection of nucleotides (LOD ~ 0.1 fmol) by amidation of *N*,*N*-dimethylaniline with a phosphate group. Han et al. developed derivatization with 3-amino-9-ethylcarbazole to increase the MS intensities with LODs of sugars and their phosphorylated products ranging from 0.06–1.37 pmol [206]. Liu et al. developed a diazo reagent labeling strategy, which showed high specificity and efficiency for the analysis of nucleotides. The sensitivities of 12 ribonucleotides increased by 12- to 174-fold with the LODs ranging from 0.07 to 0.41 fmol, which can realize the detection of these metabolites with only 8 cells [207]. In fact, without multiple phosphate groups, nucleotides with aromatic rings would have great retention behavior. Li et al. developed easy and simple derivatization with (trimethylsily)diazomethane, which only transformed phosphate groups to their esters [208]. This reaction only took 3 min and the LOQs of dCMP, dCDP and dCTP are 0.0125, 0.0625 and 0.25 pmol, respectively. The esterification of phosphate can reduce the activity of high-energy bonds of triphosphorylated metabolites, such as ATP. The stability of these metabolites can be improved and it would be benefit for long-term analysis.

5.3 Derivatization of Carbonyl Containing Metabolites

Carbonyl containing metabolites include sugars, aldehyde, ketones, keto acids and so on. Most of the derivatization of saccharides take the approach of reacting aldehyde and ketone with hydrazine and *O*-hydroxylamine to form hydrazones and osazones [167, 209, 210], as shown in Scheme 5. *N*-Hydroxylamine is prone to Hoffmann rearrangement, hence subjected to structural confirmation; therefore, the experiments are carried out mostly using *O*-hydroxylamine. However, more than 99% of the sugars in aqueous exist as hemiacetal so that the derivatizing aldehyde group will take a longer time to promote the opening of the sugar ring.



Scheme 5. Derivatization of Carbonyl group in LC–MS analysis

Zhang et al. [211] proposed a method of periodic acid oxidation to assist the derivatization of sugars, however, the products are complex because the aldehyde groups at three positions could be oxidized simultaneously. The development of open-loop strategies for sugars detection in mild conditions remains a huge challenge. It is worth noting that isomers of saccharides have different spatial structures of diols so that developing chiral probes for diol derivatization could open up new avenues for the detection of sugars.

Chemical labeling is also used for high-sensitive untargeted profiling in recent years. Although non-derivatized untargeted profiling has high coverage and no preference to metabolite detection, hydrophilic metabolites, especially polycarboxylic acids and nucleotides are low response in the negative mode of HRMS. In addition, some metabolites with low concentration in body fluids are often undetected in the untargeted analysis. The derivatization assisted profiling strategies to provide better detection for these metabolites by employing isotope labeling technique. Zhao et al. [212] use ^{12}C- and ^{13}C-dansylhydrazine to profile carboxyl metabolites by searching isotope ion pairs of metabolites. A total of 2266 peak pairs or metabolites were detected and positive identification of metabolites can also be achieved by the combination with neural loss scan and fragment ions scan.

Most derivatization methods can only be functionalized with a single chemical group, and there has been no derivatization technology that could be universally applicable to all hydrophilic metabolites. Therefore, derivatization remained to be further improved to be suitable for high-coverage untargeted profiling and biomarker discovery. A combination of different labeling methods in an integrated approach could provide a solution for the high coverage of detection required for untargeted profiling. Yuan et al. have used four derivatization reagents with their deuterium labeling standards (total 8 reagents) to the untargeted analysis of amine metabolites, carbonyl metabolites, carboxyl metabolites, and thiol metabolites. Over 2300 potential metabolites with 1388 positively or putatively identified were detected in fecal samples [213]. Zhao et al. also used a four-channel chemical isotope labeling approach, covering amine, phenol, carbonyl, carboxyl and hydroxyl metabolites, further increased the range of metabolites, and a total of 7431 peaks were detected [214].

However, the parallel labeling reaction might induce interferences between each other, therefore labeling efficiency needs to be considered. Additionally, many metabolites have multiple functional groups, such as amino acids, nucleotides, which brings more complexity for identification and filtering. Isotope-labeling profiling also needs higher resolution mass spectrometers to increase the accuracy of peak alignments, however, these types of instruments could have relatively lower scan speed, posing difficulties for identification with MS/MS. It is therefore important to point out that chemical labeling assisted untargeted profiling needs much-devoted attention in the future.

6 Conclusion

The hydrophilic metabolites with high polarity in nature play an important role in life activities. For such small polar metabolites, analysis is often difficult due to its weak hydrophobicity, low ionization, wide concentration range, and high sample complexity. Major efforts have been made in the identification and quantification of hydrophilic metabolites, including non-labeling and chemical labeling approaches. In non-labeling strategies, new chromatographic technologies have been used to enhance the separation capability. HILIC is the most important method in separating hydrophilic metabolites, especially it can couple with RPLC to realize full coverage of both hydrophilic and hydrophobic metabolites and lipids. However, the stability of retention time is still a problem and the peak shape of nucleotides in HILIC method are not satisfied. CE–MS is an essential approach that can realize single-cell detection and in situ analysis. It can detect polar metabolites, even ionized metabolites simultaneously with nano-level samples. IC–MS is similar as CE–MS which are capable for detection of strong ionized metabolites. The development of the capillary column and ion suppressor can enhance the separation of isomeric polar analytes and increase MS signals. However, CE–MS and IC–MS need opposite elutes or columns for the analysis of cationic and anionic metabolites; the low flow rate leads to low efficiency and long runtime, which are not suitable for analysis of large-scale samples. These chromatography technologies could not enhance the response in mass spectrometric analysis, which prompts the development of chemical labeling methods. Chemical labeling strategies use derivatization reagents to transform these small metabolites to aromatic derivatives with high-retention in LC and

high-sensitivity in MS. Special derivatization reagents have been designed to react with metabolites containing different functional groups, including amines, carboxyl, etc. Finally, chemical labeling methods have great potential for untargeted profiling, however, high throughput, high coverage and high-efficiency derivatization strategies are still lacking and hence urgently needed.

Author Contributions HQ, WY, and TH had the idea and designed the review. HQ performed literature research, data analysis and drafted the manuscript. WY devised this manuscript.

Funding This work was supported by grant from the National Key R&D Program of China (2017YFC0906800).

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Huang L, Kim D, Liu X, Myers CR, Locasale JW. Estimating relative changes of metabolic fluxes. PLoS Comput Biol. 2014;10(11):e1003958. https://doi.org/10.1371/journal.pcbi.1003958.
2. Weindl D, Cordes T, Battello N, Sapcariu SC, Dong X, Wegner A, et al. Bridging the gap between non-targeted stable isotope labeling and metabolic flux analysis. Cancer Metab. 2016;4:10. https://doi.org/10.1186/s40170-016-0150-z.
3. Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, et al. Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature. 2006;440(7087):1073–7. https://doi.org/10.1038/nature04648.
4. Kim HK, Choi YH, Verpoorte R. NMR-based metabolomic analysis of plants. Nat Protoc. 2010;5(3):536–49. https://doi.org/10.1038/nprot.2009.237.
5. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, et al. The human urine metabolome. PLoS One. 2013;8(9):e73076. https://doi.org/10.1371/journal.pone.0073076.
6. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, et al. The human serum metabolome. PLoS One. 2011;6(2):e16957. https://doi.org/10.1371/journal.pone.0016957.
7. Buszewski B, Noga S. Hydrophilic interaction liquid chromatography (HILIC)—a powerful separation technique. Anal Bioanal Chem. 2011;402(1):231–47. https://doi.org/10.1007/s00216-011-5308-5.
8. Marrubini G, Appelblad P, Maietta M, Papetti A. Hydrophilic interaction chromatography in food matrices analysis: an updated review. Food Chem. 2018;257:53–66. https://doi.org/10.1016/j.foodchem.2018.03.008.
9. Stolz A, Jooß K, Höcker O, Römer J, Schlecht J, Neusüß C. Recent advances in capillary electrophoresis–mass spectrometry: instrumentation, methodology and applications. Electrophoresis. 2019;40(1):79–112. https://doi.org/10.1002/elps.201800331.
10. Cajka T, Fiehn O. Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. Anal Chem. 2015;88(1):524–45. https://doi.org/10.1021/acs.analchem.5b04491.
11. Kuehnbaum NL, Britz-McKibbin P. New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era. Chem Rev. 2013;113(4):2437–68. https://doi.org/10.1021/cr300484s.
12. Bown AW, Shelp BJ. The metabolism and functions of [gamma]-aminobutyric acid. Plant Physiol. 1997;115(1):1–5. https://doi.org/10.1104/pp.115.1.1.
13. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science (New York, NY). 2017. https://doi.org/10.1126/science.aaf9794.
14. Badawy AA, Namboodiri AM, Moffett JR. The end of the road for the tryptophan depletion concept in pregnancy and infection. Clin Sci (London, England: 1979). 2016;130(15):1327–33. https://doi.org/10.1042/cs20160153.
15. Schwarcz R, Stone TW. The kynurenine pathway and the brain: challenges, controversies and promises. Neuropharmacology. 2017;112(Pt B):237–47. https://doi.org/10.1016/j.neuropharm.2016.08.003.
16. Fazio F, Lionetto L, Curto M, Iacovelli L, Copeland CS, Neale SA, et al. Cinnabarinic acid and xanthurenic acid: two kynurenine metabolites that interact with metabotropic glutamate receptors. Neuropharmacology. 2017;112(Pt B):365–72. https://doi.org/10.1016/j.neuropharm.2016.06.020.
17. Lee JH, Lee J. Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev. 2010;34(4):426–44. https://doi.org/10.1111/j.1574-6976.2009.00204.x.
18. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe. 2018;23(6):716–24. https://doi.org/10.1016/j.chom.2018.05.003.
19. Fuchs D, Avanzas P, Arroyo-Espliguero R, Jenny M, Consuegra-Sanchez L, Kaski JC. The role of neopterin in atherogenesis and cardiovascular risk assessment. Curr Med Chem. 2009;16(35):4644–53. https://doi.org/10.2174/092986709789878247.
20. Wang Y, Liu H, McKenzie G, Witting PK, Stasch JP, Hahn M, et al. Kynurenine is an endothelium-derived relaxing factor produced during inflammation. Nat Med. 2010;16(3):279–85. https://doi.org/10.1038/nm.2092.
21. Kvist E, Sjolin KE, Iversen J, Nyholm K. Urinary excretion patterns of pseudouridine and beta-aminoisobutyric acid in patients with tumours of the urinary bladder. Scand J Urol Nephrol. 1993;27(1):45–53.
22. Chou RH, Chen CY, Chen IC, Huang HL, Lu YW, Kuo CS, et al. Trimethylamine *N*-oxide, circulating endothelial progenitor cells, and endothelial function in patients with stable angina. Sci Rep. 2019;9(1):4249. https://doi.org/10.1038/s41598-019-40638-y.
23. Makki K, Deehan EC, Walter J, Backhed F. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe. 2018;23(6):705–15. https://doi.org/10.1016/j.chom.2018.05.012.
24. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. 2016;165(6):1332–455. https://doi.org/10.1016/j.cell.2016.05.041.
25. Doucette CD, Schwab DJ, Wingreen NS, Rabinowitz JD. alpha-Ketoglutarate coordinates carbon and nitrogen utilization via enzyme I inhibition. Nat Chem Biol. 2011;7(12):894–901. https://doi.org/10.1038/nchembio.685.
26. Williams HE. Oxalic acid and the hyperoxaluric syndromes. Kidney Int. 1978;13(5):410–7.
27. Ryan DG, Murphy MP, Frezza C, Prag HA, Chouchani ET, O'Neill LA, et al. Coupling Krebs cycle metabolites to signalling in immunity and cancer. Nat Metab. 2018;1(1):16–33. https://doi.org/10.1038/s42255-018-0014-7.
28. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic

and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity. 2015;42(3):419–30. https://doi.org/10.1016/j.immuni.2015.02.005.
29. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature. 2013;496(7444):238–42. https://doi.org/10.1038/nature11986.
30. He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature. 2004;429(6988):188–93. https://doi.org/10.1038/nature02488.
31. Strelko CL, Lu W, Dufort FJ, Seyfried TN, Chiles TC, Rabinowitz JD, et al. Itaconic acid is a mammalian metabolite induced during macrophage activation. J Am Chem Soc. 2011;133(41):16386–9. https://doi.org/10.1021/ja2070889.
32. Zhu X, Chen L, Wu J, Tang H, Wang Y. *Salmonella typhimurium* infection reduces *Schistosoma japonicum* worm burden in mice. Sci Rep. 2017;7(1):1349. https://doi.org/10.1038/s41598-017-00992-1.
33. Salamanca-Cardona L, Shah H, Poot AJ, Correa FM, Di Gialleonardo V, Lui H, et al. In vivo imaging of glutamine metabolism to the oncometabolite 2-hydroxyglutarate in IDH1/2 mutant tumors. Cell Metab. 2017;26(6):830–41.e3. https://doi.org/10.1016/j.cmet.2017.10.001.
34. Knowles JR. Enzyme-catalyzed phosphoryl transfer reactions. Annu Rev Biochem. 1980;49:877–919. https://doi.org/10.1146/annurev.bi.49.070180.004305.
35. Wettschureck N, Offermanns S. Mammalian G proteins and their cell type specific functions. Physiol Rev. 2005;85(4):1159–204. https://doi.org/10.1152/physrev.00003.2005.
36. Freitas FZ, de Paula RM, Barbosa LC, Terenzi HF, Bertolini MC. cAMP signaling pathway controls glycogen metabolism in *Neurospora crassa* by regulating the glycogen synthase gene expression and phosphorylation. Fungal Genet Biol FGB. 2010;47(1):43–52. https://doi.org/10.1016/j.fgb.2009.10.011.
37. Khaled M, Larribere L, Bille K, Aberdam E, Ortonne JP, Ballotti R, et al. Glycogen synthase kinase 3beta is activated by cAMP and plays an active role in the regulation of melanogenesis. J Biol Chem. 2002;277(37):33690–7. https://doi.org/10.1074/jbc.M202939200.
38. Smith RS, Weitz CJ, Araneda RC. Excitatory actions of noradrenaline and metabotropic glutamate receptor activation in granule cells of the accessory olfactory bulb. J Neurophysiol. 2009;102(2):1103–14. https://doi.org/10.1152/jn.91093.2008.
39. Dell'Acqua ML, Carroll RC, Peralta EG. Transfected m2 muscarinic acetylcholine receptors couple to G alpha i2 and G alpha i3 in Chinese hamster ovary cells. Activation and desensitization of the phospholipase C signaling pathway. J Biol Chem. 1993;268(8):5676–85.
40. Li M, Zhou X, Wang S, Michailidis I, Gong Y, Su D, et al. Structure of a eukaryotic cyclic-nucleotide-gated channel. Nature. 2017;542(7639):60–5. https://doi.org/10.1038/nature20819.
41. Gross OP, Pugh EN Jr, Burns ME. Calcium feedback to cGMP synthesis strongly attenuates single-photon responses driven by long rhodopsin lifetimes. Neuron. 2012;76(2):370–82. https://doi.org/10.1016/j.neuron.2012.07.029.
42. Michalakis S, Becirovic E, Biel M. Retinal cyclic nucleotide-gated channels: from pathophysiology to therapy. Int J Mol Sci. 2018. https://doi.org/10.3390/ijms19030749.
43. Choi M, Jeon YK, Sun CH, Yun HS, Hong J, Shin DY, et al. RTK-RAS pathway mutation is enriched in myeloid sarcoma. Blood Cancer J. 2018;8(5):43. https://doi.org/10.1038/s41408-018-0083-6.
44. Czerwoniec A, Dunin-Horkawicz S, Purta E, Kaminska KH, Kasprzak JM, Bujnicki JM, et al. MODOMICS: a database of RNA modification pathways. 2008 update. Nucleic Acids Res. 2009;37(Database issue):D118–D12121. https://doi.org/10.1093/nar/gkn710.
45. Urbonavicius J, Qian Q, Durand JM, Hagervall TG, Bjork GR. Improvement of reading frame maintenance is a common function for several tRNA modifications. EMBO J. 2001;20(17):4863–73. https://doi.org/10.1093/emboj/20.17.4863.
46. Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, et al. Innate immune and chemically triggered oxidative stress modifies translational fidelity. Nature. 2009;462(7272):522–6. https://doi.org/10.1038/nature08576.
47. Chung FL, Young R, Hecht SS. Formation of cyclic 1, N2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Can Res. 1984;44(3):990–5.
48. Nath RG, Ocando JE, Richie JP Jr, Chung FL. Effect of glutathione depletion on exocyclic adduct levels in the liver DNA of F344 rats. Chem Res Toxicol. 1997;10(11):1250–3. https://doi.org/10.1021/tx9701079.
49. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science (New York, NY). 2009;324(5929):929–30. https://doi.org/10.1126/science.1169786.
50. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science (New York, NY). 2009;324(5929):930–5. https://doi.org/10.1126/science.1170116.
51. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science (New York, NY). 2011;333(6047):1300–3. https://doi.org/10.1126/science.1210597.
52. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science (New York, NY). 2011;333(6047):1303–7. https://doi.org/10.1126/science.1210944.
53. Intlekofer AM, Finley LWS. Metabolic signatures of cancer cells and stem cells. Nat Metab. 2019;1(2):177–88. https://doi.org/10.1038/s42255-019-0032-0.
54. Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci. 2019;20(3):148–60. https://doi.org/10.1038/s41583-019-0132-6.
55. Wamelink MM, Smith DE, Jansen EE, Verhoeven NM, Struys EA, Jakobs C. Detection of transaldolase deficiency by quantification of novel seven-carbon chain carbohydrate biomarkers in urine. J Inherit Metab Dis. 2007;30(5):735–42. https://doi.org/10.1007/s10545-007-0590-2.
56. Zhang Q, Piston DW, Goodman RH. Regulation of corepressor function by nuclear NADH. Science (New York, NY). 2002;295(5561):1895–7. https://doi.org/10.1126/science.1069300.
57. Veech RL, Eggleston LV, Krebs HA. The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm of rat liver. Biochem J. 1969;115(4):609–19. https://doi.org/10.1042/bj1150609a.
58. Berger F, Ramirez-Hernandez MH, Ziegler M. The new life of a centenarian: signalling functions of NAD(P). Trends Biochem Sci. 2004;29(3):111–8. https://doi.org/10.1016/j.tibs.2004.01.007.
59. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. Nature. 2003;423(6936):181–5. https://doi.org/10.1038/nature01578.
60. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. Science (New York, NY). 2000;289(5487):2126–8.

61. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Cohen H, Lin SS, et al. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. J Biol Chem. 2002;277(21):18881–900. https://doi.org/10.1074/jbc.M111773200.
62. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem. 2002;277(47):45099–107. https://doi.org/10.1074/jbc.M205670200.
63. Petersen OH, Cancela JM. New Ca^{2+}-releasing messengers: are they important in the nervous system? Trends Neurosci. 1999;22(11):488–95.
64. Churchill GC, Okada Y, Thomas JM, Genazzani AA, Patel S, Galione A. NAADP mobilizes Ca(2+) from reserve granules, lysosome-related organelles, in sea urchin eggs. Cell. 2002;111(5):703–8.
65. Galione A, Patel S, Churchill GC. NAADP-induced calcium release in sea urchin eggs. Biol Cell. 2000;92(3–4):197–204.
66. Hartman SC, Buchanan JM. Biosynthesis of the purines. XXVI. The identification of the formyl donors of the transformylation reactions. J Biol Chem. 1959;234(7):1812–6.
67. Xue J-H, Chen G-D, Hao F, Chen H, Fang Z, Chen F-F, et al. A vitamin-C-derived DNA modification catalysed by an algal TET homologue. Nature. 2019. https://doi.org/10.1038/s41586-019-1160-0.
68. Fritz IB, Yue KT. Long-chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. J Lipid Res. 1963;4:279–88.
69. Jones LL, McDonald DA, Borum PR. Acylcarnitines: role in brain. Prog Lipid Res. 2010;49(1):61–75. https://doi.org/10.1016/j.plipres.2009.08.004.
70. Stanley CA. Carnitine deficiency disorders in children. Ann N Y Acad Sci. 2004;1033:42–51. https://doi.org/10.1196/annals.1320.004.
71. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009;9(4):311–26. https://doi.org/10.1016/j.cmet.2009.02.002.
72. Bene J, Hadzsiev K, Melegh B. Role of carnitine and its derivatives in the development and management of type 2 diabetes. Nutr Diabetes. 2018;8(1):8. https://doi.org/10.1038/s41387-018-0017-1.
73. Bernardini I, Rizzo WB, Dalakas M, Bernar J, Gahl WA. Plasma and muscle free carnitine deficiency due to renal Fanconi syndrome. J Clin Investig. 1985;75(4):1124–30. https://doi.org/10.1172/jci111806.
74. Calvani M, Benatti P, Mancinelli A, D'Iddio S, Giordano V, Koverech A, et al. Carnitine replacement in end-stage renal disease and hemodialysis. Ann N Y Acad Sci. 2004;1033:52–66. https://doi.org/10.1196/annals.1320.005.
75. Day CR, Kempson SA. Betaine chemistry, roles, and potential use in liver disease. Biochem Biophys Acta. 2016;1860(6):1098–106. https://doi.org/10.1016/j.bbagen.2016.02.001.
76. Tanaka K, West-Dull A, Hine DG, Lynn TB, Lowe T. Gas-chromatographic method of analysis for urinary organic acids. II. Description of the procedure, and its application to diagnosis of patients with organic acidurias. Clin Chem. 1980;26(13):1847–53.
77. Milkovska-Stamenova S, Schmidt R, Frolov A, Birkemeyer C. GC–MS method for the quantitation of carbohydrate intermediates in glycation systems. J Agric Food Chem. 2015;63(25):5911–9. https://doi.org/10.1021/jf505757m.
78. Kvitvang HF, Andreassen T, Adam T, Villas-Boas SG, Bruheim P. Highly sensitive GC/MS/MS method for quantitation of amino and nonamino organic acids. Anal Chem. 2011;83(7):2705–11. https://doi.org/10.1021/ac103245b.
79. El-Sayed MS, George KP, Dyson K. The influence of blood sampling site on lactate concentration during submaximal exercise at 4 mmol · 1–1 lactate level. Eur J Appl Physiol. 1993;67(6):518–22. https://doi.org/10.1007/BF00241648.
80. Pyne DB, Boston T, Martin DT, Logan A. Evaluation of the Lactate Pro blood lactate analyser. Eur J Appl Physiol. 2000;82(1):112–6. https://doi.org/10.1007/s004210050659.
81. da Silva G. Decomposition of pyruvic acid on the ground-state potential energy surface. J Phys Chem A. 2016;120(2):276–83. https://doi.org/10.1021/acs.jpca.5b10078.
82. Tewari YB, Goldberg RN, Advani JV. Thermodynamics of the disproportionation of adenosine 5′-diphosphate to adenosine 5′-triphosphate and adenosine 5′-monophosphate, II. Experimental data. Biophys Chem. 1991;40(3):263–76.
83. Gajewski E, Steckler DK, Goldberg RN. Thermodynamics of the hydrolysis of adenosine 5′-triphosphate to adenosine 5′-diphosphate. J Biol Chem. 1986;261(27):12733–7.
84. Xu YF, Lu W, Rabinowitz JD. Avoiding misannotation of in-source fragmentation products as cellular metabolites in liquid chromatography–mass spectrometry-based metabolomics. Anal Chem. 2015;87(4):2273–81. https://doi.org/10.1021/ac504118y.
85. Podolec P, Szabo AH, Blasko J, Kubinec R, Gorova R, Visnovsky J, et al. Direct silylation of *Trypanosoma brucei* metabolites in aqueous samples and their GC–MS/MS analysis. J Chromatogr B Anal Technol Biomed Life Sci. 2014;967:134–8. https://doi.org/10.1016/j.jchromb.2014.07.023.
86. Schummer C, Delhomme O, Appenzeller BM, Wennig R, Millet M. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. Talanta. 2009;77(4):1473–82. https://doi.org/10.1016/j.talanta.2008.09.043.
87. Avula SGC, Belovich JM, Xu Y. Determination of fatty acid methyl esters derived from algae *Scenedesmus dimorphus* biomass by GC-MS with one-step esterification of free fatty acids and transesterification of glycerolipids. J Sep Sci. 2017;40(10):2214–27. https://doi.org/10.1002/jssc.201601336.
88. Xu Z, Harvey K, Pavlina T, Dutot G, Zaloga G, Siddiqui R. An improved method for determining medium- and long-chain FAMEs using gas chromatography. Lipids. 2010;45(2):199–208. https://doi.org/10.1007/s11745-009-3382-7.
89. Li H, An Y, Zhang L, Lei H, Zhang L, Wang Y, et al. Combined NMR and GC–MS analyses revealed dynamic metabolic changes associated with the carrageenan-induced rat pleurisy. J Proteome Res. 2013;12(12):5520–34. https://doi.org/10.1021/pr400440d.
90. Amer B, Nebel C, Bertram HC, Mortensen G, Dalsgaard TK. Direct derivatization vs aqueous extraction methods of fecal free fatty acids for GC–MS analysis. Lipids. 2015;50(7):681–9. https://doi.org/10.1007/s11745-015-4029-5.
91. Tsikas D, Rothmann S, Schneider JY, Gutzki F-M, Beckmann B, Frölich JC. Simultaneous GC–MS/MS measurement of malondialdehyde and 4-hydroxy-2-nonenal in human plasma: effects of long-term L-arginine administration. Anal Biochem. 2017;524:31–44. https://doi.org/10.1016/j.ab.2016.08.009.
92. Kang H-I, Shin H-S. Sensitive determination of glutaraldehyde in environmental water by derivatization and gas chromatography–mass spectrometry. Anal Methods. 2016;8(15):3216–23. https://doi.org/10.1039/c5ay02798e.
93. Xu F, Zou L, Ong CN. Multiorigination of chromatographic peaks in derivatized GC/MS metabolomics: a confounder that influences metabolic pathway interpretation. J Proteome Res. 2009;8(12):5657–65. https://doi.org/10.1021/pr900738b.
94. Ghosson H, Schwarzenberg A, Jamois F, Yvin J-C. Simultaneous untargeted and targeted metabolomics profiling of underivatized primary metabolites in sulfur-deficient barley by ultra-high

performance liquid chromatography-quadrupole/time-of-flight mass spectrometry. Plant Methods. 2018;14:62. https://doi.org/10.1186/s13007-018-0329-0.
95. Velenosi TJ, Hennop A, Feere DA, Tieu A, Kucey AS, Kyriacou P, et al. Untargeted plasma and tissue metabolomics in rats with chronic kidney disease given AST-120. Sci Rep. 2016;6(1):22526. https://doi.org/10.1038/srep22526.
96. Wang S, Wang Z, Zhou L, Shi X, Xu G. Comprehensive analysis of short-, medium-, and long-chain acyl-coenzyme A by online two-dimensional liquid chromatography/mass spectrometry. Anal Chem. 2017;89(23):12902–8. https://doi.org/10.1021/acs.analchem.7b03659.
97. Alpert AJ. Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. J Chromatogr. 1990;499:177–96.
98. Hemstrom P, Irgum K. Hydrophilic interaction chromatography. J Sep Sci. 2006;29(12):1784–821.
99. Jandera P, Janas P. Recent advances in stationary phases and understanding of retention in hydrophilic interaction chromatography. A review. Anal Chim Acta. 2017;967:12–32. https://doi.org/10.1016/j.aca.2017.01.060.
100. Periat A, Debrus B, Rudaz S, Guillarme D. Screening of the most relevant parameters for method development in ultra-high performance hydrophilic interaction chromatography. J Chromatogr A. 2013;1282:72–83. https://doi.org/10.1016/j.chroma.2013.01.037.
101. Jandera P. Stationary phases for hydrophilic interaction chromatography, their characterization and implementation into multidimensional chromatography concepts. J Sep Sci. 2008;31(9):1421–37. https://doi.org/10.1002/jssc.200800051.
102. Wikberg E, Sparrman T, Viklund C, Jonsson T, Irgum K. A 2H nuclear magnetic resonance study of the state of water in neat silica and zwitterionic stationary phases and its influence on the chromatographic retention characteristics in hydrophilic interaction high-performance liquid chromatography. J Chromatogr A. 2011;1218(38):6630–8. https://doi.org/10.1016/j.chroma.2011.04.056.
103. Sonnenberg RA, Naz S, Cougnaud L, Vuckovic D. Comparison of underivatized silica and zwitterionic sulfobetaine hydrophilic interaction liquid chromatography stationary phases for global metabolomics of human plasma. J Chromatogr A. 2019;1608:460419. https://doi.org/10.1016/j.chroma.2019.460419.
104. Hsiao JJ, Potter OG, Chu TW, Yin H. Improved LC/MS methods for the analysis of metal-sensitive analytes using medronic acid as a mobile phase additive. Anal Chem. 2018;90(15):9457–64. https://doi.org/10.1021/acs.analchem.8b02100.
105. Olesti E, Rodríguez-Morató J, Gomez-Gomez A, Ramaekers JG, de la Torre R, Pozo OJ. Quantification of endogenous neurotransmitters and related compounds by liquid chromatography coupled to tandem mass spectrometry. Talanta. 2019;192:93–102. https://doi.org/10.1016/j.talanta.2018.09.034.
106. Rivetti C, Climent E, Gómez-Canela C, Barata C. Characterization of neurotransmitter profiles in *Daphnia magna* juveniles exposed to environmental concentrations of antidepressants and anxiolytic and antihypertensive drugs using liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem. 2019;411(22):5867–76. https://doi.org/10.1007/s00216-019-01968-y.
107. Zhu B, Li L, Wei H, Zhou W, Zhou W, Li F, et al. A simultaneously quantitative profiling method for 40 endogenous amino acids and derivatives in cell lines using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. Talanta. 2020. https://doi.org/10.1016/j.talanta.2019.120256.
108. Xiang L, Wei J, Tian XY, Wang B, Chan W, Li S, et al. Comprehensive analysis of acylcarnitine species in db/db mouse using a novel method of high-resolution parallel reaction monitoring reveals widespread metabolic dysfunction induced by diabetes. Anal Chem. 2017;89(19):10368–75. https://doi.org/10.1021/acs.analchem.7b02283.
109. Wang M, Yang R, Mu H, Zeng J, Zhang T, Zhou W, et al. A simple and precise method for measurement of serum free carnitine and acylcarnitines by isotope dilution HILIC–ESI–MS/MS. Int J Mass Spectrom. 2019. https://doi.org/10.1016/j.ijms.2019.116208.
110. Steuer C, Schutz P, Bernasconi L, Huber AR. Simultaneous determination of phosphatidylcholine-derived quaternary ammonium compounds by a LC–MS/MS method in human blood plasma, serum and urine samples. J Chromatogr B Anal Technol Biomed Life Sci. 2016;1008:206–11. https://doi.org/10.1016/j.jchromb.2015.12.002.
111. Li X, Wong CC, Tang Z, Wu J, Li S, Qian Y, et al. Determination of amino acids in colon cancer cells by using UHPLC-MS/MS and [U-(13)C5]-glutamine as the isotope tracer. Talanta. 2017;162:285–92. https://doi.org/10.1016/j.talanta.2016.10.013.
112. Prinsen H, Schiebergen-Bronkhorst BGM, Roeleveld MW, Jans JJM, de Sain-van der Velden MGM, Visser G, et al. Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. J Inherit Metab Dis. 2016;39(5):651–60. https://doi.org/10.1007/s10545-016-9935-z.
113. Mathon C, Barding GA Jr, Larive CK. Separation of ten phosphorylated mono-and disaccharides using HILIC and ion-pairing interactions. Anal Chim Acta. 2017;972:102–10. https://doi.org/10.1016/j.aca.2017.03.029.
114. Mata AT, Jorge TF, Ferreira J, do Rosario Bronze M, Branco D, Fevereiro P, et al. Analysis of low abundant trehalose-6-phosphate and related metabolites in *Medicago truncatula* by hydrophilic interaction liquid chromatography-triple quadrupole mass spectrometry. J Chromatogr A. 2016;1477:30–8. https://doi.org/10.1016/j.chroma.2016.11.031.
115. Dong M, Qin L, Ma LX, Zhao ZY, Du M, Kunihiko K, et al. Postmortem nucleotide degradation in turbot mince during chill and partial freezing storage. Food Chem. 2020;311:125900. https://doi.org/10.1016/j.foodchem.2019.125900.
116. Ambati CR, Vantaku V, Donepudi SR, Amara CS, Ravi SS, Mandalapu A, et al. Measurement of methylated metabolites using liquid chromatography–mass spectrometry and its biological application. Anal Methods. 2019;11(1):49–57. https://doi.org/10.1039/c8ay02168f.
117. Su D, Chan CTY, Gu C, Lim KS, Chionh YH, McBee ME, et al. Quantitative analysis of ribonucleoside modifications in tRNA by HPLC-coupled mass spectrometry. Nat Protoc. 2014;9:828. https://doi.org/10.1038/nprot.2014.047.
118. Liao H, Zang Q, Lv Q, Gao Y, Zhao Z, He J, et al. Development of methionine methylation profiling and relative quantification in human breast cancer cells based on metabolic stable isotope labeling. Analyst. 2019;144(13):3988–98. https://doi.org/10.1039/c9an00545e.
119. Feith A, Teleki A, Graf M, Favilli L, Takors R. HILIC-enabled 13C metabolomics strategies: comparing quantitative precision and spectral accuracy of QTOF high- and QQQ low-resolution mass spectrometry. Metabolites. 2019. https://doi.org/10.3390/metabo9040063.
120. Yuan M, Kremer DM, Huang H, Breitkopf SB, Ben-Sahra I, Manning BD, et al. Ex vivo and in vivo stable isotope labelling of central carbon metabolism and related pathways with analysis by LC–MS/MS. Nat Protoc. 2019;14(2):313–30. https://doi.org/10.1038/s41596-018-0102-x.
121. Creek DJ, Chokkathukalam A, Jankevics A, Burgess KE, Breitling R, Barrett MP. Stable isotope-assisted metabolomics for network-wide metabolic pathway elucidation. Anal Chem. 2012;84(20):8442–7. https://doi.org/10.1021/ac3018795.

122. Ortmayr K, Schwaiger M, Hann S, Koellensperger G. An integrated metabolomics workflow for the quantification of sulfur pathway intermediates employing thiol protection with *N*-ethyl maleimide and hydrophilic interaction liquid chromatography tandem mass spectrometry. Analyst. 2015;140(22):7687–95. https://doi.org/10.1039/c5an01629k.
123. Schwaiger M, Schoeny H, El Abiead Y, Hermann G, Rampler E, Koellensperger G. Merging metabolomics and lipidomics into one analytical run. Analyst. 2019;144(1):220–9. https://doi.org/10.1039/c8an01219a.
124. Liu W, Song Q, Cao Y, Zhao Y, Huo H, Wang Y, et al. Advanced liquid chromatography-mass spectrometry enables merging widely targeted metabolomics and proteomics. Anal Chim Acta. 2019;1069:89–97. https://doi.org/10.1016/j.aca.2019.04.013.
125. Contrepois K, Jiang L, Snyder M. 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 Proteom. 2015;14(6):1684–95. https://doi.org/10.1074/mcp.M114.046508.
126. Frost NW, Jing M, Bowser MT. Capillary electrophoresis. Anal Chem. 2010;82(12):4682–98. https://doi.org/10.1021/ac101151k.
127. Zuskova I, Novotna A, Vcelakova K, Gas B. Determination of limiting mobilities and dissociation constants of 21 amino acids by capillary zone electrophoresis at very low pH. J Chromatogr B Anal Technol Biomed Life Sci. 2006;841(1–2):129–34. https://doi.org/10.1016/j.jchromb.2006.03.015.
128. Harada S, Hirayama A, Chan Q, Kurihara A, Fukai K, Iida M, et al. Reliability of plasma polar metabolite concentrations in a large-scale cohort study using capillary electrophoresis–mass spectrometry. PLoS One. 2018;13(1):e0191230. https://doi.org/10.1371/journal.pone.0191230.
129. Hirayama A, Sugimoto M, Suzuki A, Hatakeyama Y, Enomoto A, Harada S, et al. Effects of processing and storage conditions on charged metabolomic profiles in blood. Electrophoresis. 2015;36(18):2148–55. https://doi.org/10.1002/elps.201400600.
130. Saoi M, Percival M, Nemr C, Li A, Gibala M, Britz-McKibbin P. Characterization of the human skeletal muscle metabolome for elucidating the mechanisms of bicarbonate ingestion on strenuous interval exercise. Anal Chem. 2019;91(7):4709–18. https://doi.org/10.1021/acs.analchem.9b00149.
131. Huang T, Armbruster M, Lee R, Hui DS, Edwards JL. Metabolomic analysis of mammalian cells and human tissue through one-pot two stage derivatizations using sheathless capillary electrophoresis–electrospray ionization–mass spectrometry. J Chromatogr A. 2018;1567:219–25. https://doi.org/10.1016/j.chroma.2018.07.007.
132. DiBattista A, McIntosh N, Lamoureux M, Al-Dirbashi OY, Chakraborty P, Britz-McKibbin P. Metabolic signatures of cystic fibrosis identified in dried blood spots for newborn screening without carrier identification. J Proteome Res. 2019;18(3):841–54. https://doi.org/10.1021/acs.jproteome.8b00351.
133. Cieslarova Z, Magaldi M, Barros LA, do Lago CL, Oliveira DR, Fonseca FAH, et al. Capillary electrophoresis with dual diode array detection and tandem mass spectrometry to access cardiovascular biomarkers candidates in human urine: trimethylamine-*N*-oxide and L-carnitine. J Chromatogr A. 2019;1583:136–42. https://doi.org/10.1016/j.chroma.2018.10.005.
134. Moldovan R-C, Bodoki E, Servais A-C, Chankvetadze B, Crommen J, Oprean R, et al. Capillary electrophoresis–mass spectrometry of derivatized amino acids for targeted neurometabolomics—pH mediated reversal of diastereomer migration order. J Chromatogr A. 2018;1564:199–206. https://doi.org/10.1016/j.chroma.2018.06.030.
135. Portero EP, Nemes P. Dual cationic–anionic profiling of metabolites in a single identified cell in a live *Xenopus laevis* embryo by microprobe CE–ESI–MS. Analyst. 2019;144(3):892–900. https://doi.org/10.1039/c8an01999a.
136. Binek A, Rojo D, Godzien J, Rupérez FJ, Nuñez V, Jorge I, et al. Flow cytometry has a significant impact on the cellular metabolome. J Proteome Res. 2018. https://doi.org/10.1021/acs.jproteome.8b00472.
137. Mateos-Vivas M, Dominguez-Alvarez J, Rodriguez-Gonzalo E, Carabias-Martinez R. Capillary electrophoresis coupled to mass spectrometry employing hexafluoro-2-propanol for the determination of nucleosides and nucleotide mono-, di- and triphosphates in baby foods. Food Chem. 2017;233:38–44. https://doi.org/10.1016/j.foodchem.2017.04.060.
138. Onjiko RM, Portero EP, Moody SA, Nemes P. In situ microprobe single-cell capillary electrophoresis mass spectrometry: metabolic reorganization in single differentiating cells in the live vertebrate (*Xenopus laevis*) embryo. Anal Chem. 2017;89(13):7069–76. https://doi.org/10.1021/acs.analchem.7b00880.
139. Nemes P, Knolhoff AM, Rubakhin SS, Sweedler JV. Metabolic differentiation of neuronal phenotypes by single-cell capillary electrophoresis–electrospray ionization–mass spectrometry. Anal Chem. 2011;83(17):6810–7. https://doi.org/10.1021/ac2015855.
140. Castro-Puyana M, Garcia-Ruiz C, Crego AL, Marina ML. Development of a CE-MS(2) method for the enantiomeric separation of L/D-carnitine: application to the analysis of infant formulas. Electrophoresis. 2009;30(2):337–48. https://doi.org/10.1002/elps.200800163.
141. Sanchez-Hernandez L, Serra NS, Marina ML, Crego AL. Enantiomeric separation of free L- and D-amino acids in hydrolyzed protein fertilizers by capillary electrophoresis tandem mass spectrometry. J Agric Food Chem. 2013;61(21):5022–30. https://doi.org/10.1021/jf4013345.
142. Duncan KD, Lanekoff I. Spatially defined surface sampling capillary electrophoresis mass spectrometry. Anal Chem. 2019;91(12):7819–27. https://doi.org/10.1021/acs.analchem.9b01516.
143. Mokaddem M, Gareil P, Belgaied JE, Varenne A. New insight into suction and dilution effects in CE coupled to MS via an ESI interface. II—dilution effect. Electrophoresis. 2009;30(10):1692–7. https://doi.org/10.1002/elps.200800480.
144. Gas B, Kenndler E. Dispersive phenomena in electromigration separation methods. Electrophoresis. 2000;21(18):3888–977. https://doi.org/10.1002/1522-2683(200012)21:18%3c3888:Aid-elps3888%3e3.0.Co;2-d.
145. Hutterer KM, Jorgenson JW. Ultrahigh-voltage capillary zone electrophoresis. Anal Chem. 1999;71(7):1293–7. https://doi.org/10.1021/ac981221e.
146. Tseng MC, Chen YR, Her GR. A beveled tip sheath liquid interface for capillary electrophoresis–electrospray ionization-mass spectrometry. Electrophoresis. 2004;25(13):2084–9. https://doi.org/10.1002/elps.200305907.
147. Hirayama A, Abe H, Yamaguchi N, Tabata S, Tomita M, Soga T. Development of a sheathless CE–ESI–MS interface. Electrophoresis. 2018;39(11):1382–9. https://doi.org/10.1002/elps.201800017.
148. Boizard F, Brunchault V, Moulos P, Breuil B, Klein J, Lounis N, et al. A capillary electrophoresis coupled to mass spectrometry pipeline for long term comparable assessment of the urinary metabolome. Sci Rep. 2016;6:34453. https://doi.org/10.1038/srep34453.
149. Drouin N, Pezzatti J, Gagnebin Y, Gonzalez-Ruiz V, Schappler J, Rudaz S. Effective mobility as a robust criterion for compound annotation and identification in metabolomics: toward a mobility-based library. Anal Chim Acta. 2018;1032:178–87. https://doi.org/10.1016/j.aca.2018.05.063.

150. Fritz JS. Factors affecting selectivity in ion chromatography. J Chromatogr A. 2005;1085(1):8–17.
151. Hu S, Wang J, Ji EH, Christison T, Lopez L, Huang Y. Targeted Metabolomic Analysis of Head and Neck Cancer Cells Using High Performance Ion Chromatography Coupled with a Q Exactive HF Mass Spectrometer. Analytical Chemistry. 2015;87(12):6371–9. https://doi.org/10.1021/acs.analchem.5b01350.
152. Karu N, Dicinoski GW, Haddad PR. Use of suppressors for signal enhancement of weakly-acidic analytes in ion chromatography with universal detection methods. TrAC Trends Anal Chem. 2012;40:119–32. https://doi.org/10.1016/j.trac.2012.07.019.
153. Petucci C, Zelenin A, Culver JA, Gabriel M, Kirkbride K, Christison TT, et al. Use of ion chromatography/mass spectrometry for targeted metabolite profiling of polar organic acids. Anal Chem. 2016;88(23):11799–803. https://doi.org/10.1021/acs.analchem.6b03435.
154. Bruggink C, Maurer R, Herrmann H, Cavalli S, Hoefler F. Analysis of carbohydrates by anion exchange chromatography and mass spectrometry. J Chromatogr A. 2005;1085(1):104–9.
155. Wang J, Schnute WC. Optimizing mass spectrometric detection for ion chromatographic analysis. I. Common anions and selected organic acids. Rapid Commun Mass Spectrom RCM. 2009;23(21):3439–47. https://doi.org/10.1002/rcm.4263.
156. Burgess K, Creek D, Dewsbury P, Cook K, Barrett MP. Semi-targeted analysis of metabolites using capillary-flow ion chromatography coupled to high-resolution mass spectrometry. Rapid Commun Mass Spectrom RCM. 2011;25(22):3447–522. https://doi.org/10.1002/rcm.5247.
157. Wang J, Christison TT, Misuno K, Lopez L, Huhmer AF, Huang Y, et al. Metabolomic profiling of anionic metabolites in head and neck cancer cells by capillary ion chromatography with orbitrap mass spectrometry. Anal Chem. 2014;86(10):5116–244. https://doi.org/10.1021/ac500951v.
158. Sun Y, Saito K, Iiji R, Saito Y. Application of ion chromatography coupled with mass spectrometry for human serum and urine metabolomics. SLAS Discov Adv Sci Drug Discov. 2019;24(7):778–86. https://doi.org/10.1177/2472555219850082.
159. Miyajima M, Zhang B, Sugiura Y, Sonomura K, Guerrini MM, Tsutsui Y, et al. Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior. Nat Immunol. 2017;18(12):1342–52. https://doi.org/10.1038/ni.3867.
160. Oka M, Hashimoto K, Yamaguchi Y, Saitoh S, Sugiura Y, Motoi Y, et al. Arl8b is required for lysosomal degradation of maternal proteins in the visceral yolk sac endoderm of mouse embryos. J Cell Sci. 2017;130(20):3568–77. https://doi.org/10.1242/jcs.200519.
161. Weng G, Liu Z, Chen J, Wang F, Pan Y, Zhang Y. Enhancing the mass spectrometry sensitivity for oligonucleotide detection by organic vapor assisted electrospray. Anal Chem. 2017;89(19):10256–63. https://doi.org/10.1021/acs.analchem.7b01695.
162. Gong L, McCullagh JSO. Comparing ion-pairing reagents and sample dissolution solvents for ion-pairing reversed-phase liquid chromatography/electrospray ionization mass spectrometry analysis of oligonucleotides. Rapid Commun Mass Spectrom. 2014;28(4):339–50. https://doi.org/10.1002/rcm.6773.
163. Luo B, Groenke K, Takors R, Wandrey C, Oldiges M. Simultaneous determination of multiple intracellular metabolites in glycolysis, pentose phosphate pathway and tricarboxylic acid cycle by liquid chromatography–mass spectrometry. J Chromatogr A. 2007;1147(2):153–64. https://doi.org/10.1016/j.chroma.2007.02.034.
164. Kiefer P, Delmotte NL, Vorholt JA. Nanoscale ion-pair reversed-phase HPLC–MS for sensitive metabolome analysis. Anal Chem. 2011;83(3):850–5. https://doi.org/10.1021/ac102445r.
165. Buescher JM, Moco S, Sauer U, Zamboni N. Ultrahigh performance liquid chromatography-tandem mass spectrometry method for fast and robust quantification of anionic and aromatic metabolites. Anal Chem. 2010;82(11):4403–12. https://doi.org/10.1021/ac100101d.
166. Lu W, Clasquin MF, Melamud E, Amador-Noguez D, Caudy AA, Rabinowitz JD. Metabolomic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand alone orbitrap mass spectrometer. Anal Chem. 2010;82(8):3212–21. https://doi.org/10.1021/ac902837x.
167. Guo L, Worth AJ, Mesaros C, Snyder NW, Glickson JD, Blair IA. Diisopropylethylamine/hexafluoroisopropanol-mediated ion-pairing ultra-high-performance liquid chromatography/mass spectrometry for phosphate and carboxylate metabolite analysis: utility for studying cellular metabolism. Rapid Commun Mass Spectrom. 2016;30(16):1835–45. https://doi.org/10.1002/rcm.7667.
168. Gong L. Comparing ion-pairing reagents and counter anions for ion-pair reversed-phase liquid chromatography/electrospray ionization mass spectrometry analysis of synthetic oligonucleotides. Rapid Commun Mass Spectrom. 2015;29(24):2402–10. https://doi.org/10.1002/rcm.7409.
169. Li H, Wickramasekara S, Nemes P. One-hour screening of adulterated heparin by simplified peroxide digestion and fast RPIP-LC-MS2. Anal Chem. 2015;87(16):8424–32. https://doi.org/10.1021/acs.analchem.5b01788.
170. Parker ET, Cleaves HJ, Bada JL, Fernández FM. Quantitation of α-hydroxy acids in complex prebiotic mixtures via liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2016;30(18):2043–51. https://doi.org/10.1002/rcm.7684.
171. Adler LN, Gomez TA, Clarke SG, Linster CL. A novel GDP-D-glucose phosphorylase involved in quality control of the nucleoside diphosphate sugar pool in *Caenorhabditis elegans* and mammals. J Biol Chem. 2011;286(24):21511–233. https://doi.org/10.1074/jbc.M111.238774.
172. Horvath C, Melander W, Molnar I, Molnar P. Enhancement of retention by ion-pair formation in liquid chromatography with nonpolar stationary phases. Anal Chem. 1977;49(14):2295–305. https://doi.org/10.1021/ac50022a048.
173. Knox JH, Laird GR. Soap chromatography—a new high-performance liquid chromatographic technique for separation of ionizable materials: dyestuff intermediates. J Chromatogr A. 1976;122:17–34. https://doi.org/10.1016/S0021-9673(00)82234-7.
174. Horvath C, Melander W, Molnar I. Liquid chromatography of ionogenic substances with nonpolar stationary phases. Anal Chem. 1977;49(1):142–54. https://doi.org/10.1021/ac50009a044.
175. Bidlingmeyer BA, Deming SN, Price WP, Sachok B, Petrusek M. Retention mechamism for reversed-phase ion-pair liquid chromatography. J Chromatogr A. 1979;186:419–34. https://doi.org/10.1016/S0021-9673(00)95264-6.
176. Kraak JC, Jonker KM, Huber JFK. Solvent-generated ion-exchange systems with anionic surfactants for rapid separations of amino acids. J Chromatogr A. 1977;142:671–88. https://doi.org/10.1016/S0021-9673(01)92076-X.
177. Cecchi T, Passamonti P. Retention mechanism for ion-pair chromatography with chaotropic reagents. J Chromatogr A. 2009;1216(10):1789–97. https://doi.org/10.1016/j.chroma.2008.10.031.
178. Coulier L, Bas R, Jespersen S, Verheij E, van der Werf MJ, Hankemeier T. Simultaneous quantitative analysis of metabolites using ion-pair liquid chromatography-electrospray ionization

mass spectrometry. Anal Chem. 2006;78(18):6573–82. https://doi.org/10.1021/ac0607616.
179. Guo K, Peng J, Zhou R, Li L. Ion-pairing reversed-phase liquid chromatography fractionation in combination with isotope labeling reversed-phase liquid chromatography–mass spectrometry for comprehensive metabolome profiling. J Chromatogr A. 2011;1218(23):3689–94. https://doi.org/10.1016/j.chroma.2011.04.024.
180. Rutters H, Mohring T, Rullkotter J, Griep-Raming J, Metzger JO. The persistent memory effect of triethylamine in the analysis of phospholipids by liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom RCM. 2000;14(2):122–3. https://doi.org/10.1002/(sici)1097-0231(20000130)14:2%3c122:Aid-rcm844%3e3.0.Co;2-r.
181. Solakyildirim K, Zhang Z, Linhardt RJ. Ultraperformance liquid chromatography with electrospray ionization ion trap mass spectrometry for chondroitin disaccharide analysis. Anal Biochem. 2010;397(1):24–8. https://doi.org/10.1016/j.ab.2009.09.031.
182. Doneanu CE, Chen W, Gebler JC. Analysis of oligosaccharides derived from heparin by ion-pair reversed-phase chromatography/mass spectrometry. Anal Chem. 2009;81(9):3485–99. https://doi.org/10.1021/ac802770r.
183. Bhattacharyya S, Solakyildirim K, Zhang Z, Linhardt RJ, Tobacman JK. Chloroquine reduces arylsulphatase B activity and increases chondroitin-4-sulphate: implications for mechanisms of action and resistance. Malar J. 2009;8:303. https://doi.org/10.1186/1475-2875-8-303.
184. Laremore TN, Leach FE 3rd, Solakyildirim K, Amster IJ, Linhardt RJ. Glycosaminoglycan characterization by electrospray ionization mass spectrometry including fourier transform mass spectrometry. Methods Enzymol. 2010;478:79–108. https://doi.org/10.1016/s0076-6879(10)78003-4.
185. Gasimli L, Stansfield HE, Nairn AV, Liu H, Paluh JL, Yang B, et al. Structural remodeling of proteoglycans upon retinoic acid-induced differentiation of NCCIT cells. Glycoconj J. 2012;30(5):497–510. https://doi.org/10.1007/s10719-012-9450-x.
186. Studzińska S, Rola R, Buszewski B. The impact of ion-pairing reagents on the selectivity and sensitivity in the analysis of modified oligonucleotides in serum samples by liquid chromatography coupled with tandem mass spectrometry. J Pharm Biomed Anal. 2017;138:146–52. https://doi.org/10.1016/j.jpba.2017.02.014.
187. Michopoulos F, Whalley N, Theodoridis G, Wilson ID, Dunkley TPJ, Critchlow SE. 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. 2014;1349:60–8. https://doi.org/10.1016/j.chroma.2014.05.019.
188. Yang S, Sadilek M, Lidstrom ME. Streamlined pentafluorophenylpropyl column liquid chromatography–tandem quadrupole mass spectrometry and global 13C-labeled internal standards improve performance for quantitative metabolomics in bacteria. J Chromatogr A. 2010;1217(47):7401–10. https://doi.org/10.1016/j.chroma.2010.09.055.
189. Qi B-L, Liu P, Wang Q-Y, Cai W-J, Yuan B-F, Feng Y-Q. Derivatization for liquid chromatography-mass spectrometry. TrAC Trends Anal Chem. 2014;59:121–32. https://doi.org/10.1016/j.trac.2014.03.013.
190. Xu C, Hu X, Wang J, Zhang Y-M, Liu X-J, Xie B-B, et al. Library of Antifouling Surfaces Derived From Natural Amino Acids by Click Reaction. ACS Applied Materials & Interfaces. 2015;7(31):17337–45. https://doi.org/10.1021/acsami.5b04520.
191. Lang K, Chin JW. Bioorthogonal reactions for labeling proteins. ACS Chem Biol. 2014;9(1):16–20. https://doi.org/10.1021/cb4009292.
192. Kong N, Zhou J, Park J, Xie S, Ramström O, Yan M. Quantitative fluorine NMR to determine carbohydrate density on glyconanomaterials synthesized from perfluorophenyl azide-functionalized silica nanoparticles by click reaction. Anal Chem. 2015;87(18):9451–8. https://doi.org/10.1021/acs.analchem.5b02507.
193. Liang Y, Jiang X, Yuan R, Zhou Y, Ji C, Yang L, et al. Metabolism-based click-mediated platform for specific imaging and quantification of cell surface sialic acids. Anal Chem. 2016;89(1):538–43. https://doi.org/10.1021/acs.analchem.6b04141.
194. Wang L, Jin Z, Wang X, Zeng S, Sun C, Pan Y. Pair of stereodynamic chiral benzylicaldehyde probes for determination of absolute configuration of amino acid residues in peptides by mass spectrometry. Anal Chem. 2017;89(22):11902–7. https://doi.org/10.1021/acs.analchem.7b03804.
195. Zhou R, Guo K, Li L. 5-Diethylamino-naphthalene-1-sulfonyl Chloride (DensCl): a novel triplex isotope labeling reagent for quantitative metabolome analysis by liquid chromatography mass spectrometry. Anal Chem. 2013;85(23):11532–9. https://doi.org/10.1021/ac403000n.
196. Zhang Q, Xu H, Liu R, Gao P, Yang X, Li P, et al. Highly sensitive quantification method for amine submetabolome based on AQC-labeled-LC-tandem-MS and multiple statistical data mining: a potential cancer screening approach. Anal Chem. 2018;90(20):11941–8. https://doi.org/10.1021/acs.analchem.8b02372.
197. Wang J, Zhou L, Lei H, Hao F, Liu X, Wang Y, et al. Simultaneous quantification of amino metabolites in multiple metabolic pathways using ultra-high performance liquid chromatography with tandem-mass spectrometry. Sci Rep. 2017;7(1):1423. https://doi.org/10.1038/s41598-017-01435-7.
198. Huang W, Lan M-D, Qi C-B, Zheng S-J, Wei S-Z, Yuan B-F, et al. Formation and determination of the oxidation products of 5-methylcytosine in RNA. Chem Sci. 2016;7(8):5495–502. https://doi.org/10.1039/c6sc01589a.
199. Eggink M, Wijtmans M, Kretschmer A, Kool J, Lingeman H, de Esch IJP, et al. Targeted LC–MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA. Anal Bioanal Chem. 2010;397(2):665–75. https://doi.org/10.1007/s00216-010-3575-1.
200. Tan B, Lu Z, Dong S, Zhao G, Kuo MS. Derivatization of the tricarboxylic acid intermediates with *O*-benzylhydroxylamine for liquid chromatography-tandem mass spectrometry detection. Anal Biochem. 2014;465:134–47. https://doi.org/10.1016/j.ab.2014.07.027.
201. Zhao S, Dawe M, Guo K, Li L. Development of high-performance chemical isotope labeling LC–MS for profiling the carbonyl submetabolome. Anal Chem. 2017;89(12):6758–65. https://doi.org/10.1021/acs.analchem.7b01098.
202. Guo K, Li L. High-performance isotope labeling for profiling carboxylic acid-containing metabolites in biofluids by mass spectrometry. Anal Chem. 2010;82(21):8789–93. https://doi.org/10.1021/ac102146g.
203. Jiang R, Jiao Y, Zhang P, Liu Y, Wang X, Huang Y, et al. Twin derivatization strategy for high-coverage quantification of free fatty acids by liquid chromatography-tandem mass spectrometry. Anal Chem. 2017. https://doi.org/10.1021/acs.analchem.7b03020.
204. Zimmermann M, Sauer U, Zamboni N. Quantification and mass isotopomer profiling of α-keto acids in central carbon metabolism. Anal Chem. 2014;86(6):3232–7. https://doi.org/10.1021/ac500472c.
205. Zeng H, Qi C-B, Liu T, Xiao H-M, Cheng Q-Y, Jiang H-P, et al. Formation and determination of endogenous methylated nucleotides in mammals by chemical labeling coupled with mass

spectrometry analysis. Anal Chem. 2017;89(7):4153–60. https://doi.org/10.1021/acs.analchem.7b00052.
206. Han J, Tschernutter V, Yang J, Eckle T, Borchers CH. Analysis of selected sugars and sugar phosphates in mouse heart tissue by reductive amination and liquid chromatography-electrospray ionization mass spectrometry. Anal Chem. 2013;85(12):5965–73. https://doi.org/10.1021/ac400769g.
207. Liu F-L, Qi C-B, Cheng Q-Y, Ding J-H, Yuan B-F, Feng Y-Q. Diazo reagent labeling with mass spectrometry analysis for sensitive determination of ribonucleotides in living organisms. Anal Chem. 2019;92(2):2301–9. https://doi.org/10.1021/acs.analchem.9b05122.
208. Li Z, Zhang H-X, Li Y, Lam CWK, Wang C-Y, Zhang W-J, et al. Method for quantification of ribonucleotides and deoxyribonucleotides in human cells using (trimethylsilyl)diazomethane derivatization followed by liquid chromatography-tandem mass spectrometry. Anal Chem. 2018;91(1):1019–26. https://doi.org/10.1021/acs.analchem.8b04281.
209. Szarka S, Prokai-Tatrai K, Prokai L. Application of screening experimental designs to assess chromatographic isotope effect upon isotope-coded derivatization for quantitative liquid chromatography–mass spectrometry. Anal Chem. 2014;86(14):7033–40. https://doi.org/10.1021/ac501309s.
210. Tie C, Hu T, Jia Z-X, Zhang J-L. Derivatization strategy for the comprehensive characterization of endogenous fatty aldehydes using HPLC-multiple reaction monitoring. Anal Chem. 2016;88(15):7762–8. https://doi.org/10.1021/acs.analchem.6b01756.
211. Zhang Y, Yu M, Zhang C, Ma W, Zhang Y, Wang C, et al. Highly selective and ultra fast solid-phase extraction of n-glycoproteome by oxime click chemistry using aminooxy-functionalized magnetic nanoparticles. Anal Chem. 2014;86(15):7920–4. https://doi.org/10.1021/ac5018666.
212. Zhao S, Li L. Dansylhydrazine isotope labeling LC–MS for comprehensive carboxylic acid submetabolome profiling. Anal Chem. 2018;90(22):13514–22. https://doi.org/10.1021/acs.analchem.8b03435.
213. Yuan B-F, Zhu Q-F, Guo N, Zheng S-J, Wang Y-L, Wang J, et al. Comprehensive profiling of fecal metabolome of mice by integrated chemical isotope labeling-mass spectrometry analysis. Anal Chem. 2018;90(5):3512–20. https://doi.org/10.1021/acs.analchem.7b05355.
214. Zhao S, Li H, Han W, Chan W, Li L. Metabolomic coverage of chemical-group-submetabolome analysis: group classification and four-channel chemical isotope labeling LC–MS. Anal Chem. 2019;91(18):12108–15. https://doi.org/10.1021/acs.analchem.9b03431.