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Probing the polar metabolome by UHPLC-MS

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ABSTRACT

Metabolomics is an interdisciplinary field with applications in many areas. Analytes include metabolites involved in pathways such as glycolysis, amino acid metabolism, the Krebs cycle, etc. However, the metabolites involved in these biosynthetic pathways are typically highly polar molecules, which represent a major challenge for chromatographic analysis. Whilst there have been significant efforts to address the difficulties involved in the determination of polar metabolites the comprehensive profiling of the polar metabolome remains problematic. The current review summarizes current approaches, advances and trends in the use of liquid chromatography/mass spectrometry as it attempts to address the major instrumental and intellectual challenges involved in mapping the polar metabolome.

1. Introduction: metabolomics and the polar metabolome

Metabolomics is focused on the analysis of the metabolites found in biological samples such as plant or animal tissues, cells, cell media, and biological fluids. These metabolites (molecular masses typically less than 1000 Da), have a wide range of physicochemical properties and are present in quantities ranging from fmol to mmol, making global analysis difficult [1,2]. The perfect method for metabolomics research would provide rapid analysis, require minimal sample preparation, and offer unbiased results for all of the metabolites contained in the sample, regardless of concentration or molecular properties [3]. Furthermore, the detection system's high structural information content would allow the identification of possible biomarkers. Currently, such methods do not exist and often several analyses are needed to maximize metabolome coverage.

At the present time, liquid chromatography-mass spectrometry (LC/MS), predominantly ultra (high) performance liquid chromatography (U(H)PLC), is the most widely used approach for analysis in metabolic phenotyping, giving high throughput and coverage of metabolites over a wide range of classes and polarities [3], with reversed-phase (RPLC) separations dominating applications. However, polar/ionic compounds (e.g., many carbohydrates, organic acids, biogenic amines, saccharides, nucleotides and most amino acids) are often poorly retained by RPLC. This represents a major limitation as many important biochemical pathways, such as e.g., central carbon metabolism, are composed almost entirely of polar metabolites. The poor retention of polar analytes in RPLC systems means many compounds elute near the solvent front and this results in coelution, ion suppression and identification issues. Because of such limitations other methods using e.g., hydrophilic interaction chromatography (HILIC), ion-exchange LC (IEC), aqueous normal phase chromatography (ANP), or "mixed-mode"

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Abbreviations

2D	Two-Dimensional
AF	Ammonium Formate
ANP	Aqueous Normal-Phase
AX	Anion-Exchange
Cap	Capillary
CCS	Collision Cross Section
CX	Cation-Exchange
DEEMM	Diethyl Ethoxymethylenemalonate
ESI	Electrospray Ionization
FA	Formic Acid
GC	Gas Chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
IEX	Ion-Exchange Chromatography
IPC	Ion-Pair Chromatography
IPR	Ion-Pair Reagent
LC	Liquid Chromatography
MetID	Metabolite Identification
MMC	Mixed-mode Chromatography
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NFPA	Nonafluoropentanoic Acid
NLS	Neutral Loss Scan
PFPA	Pentafluoropropionic Acid
RP	Reversed-Phase
RPLC	Reversed-Phase Liquid Chromatography
SAX	Strong Anion-Exchange
SCX	Strong Cation-Exchange
TBA	Tributylamine
TCA	Tricarboxylic Acid
TEA	Triethylamine
TOF	Time of Flight
UHPLC	Ultrahigh Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
WAX	Weak Anion-Exchange
WCX	Weak Cation-Exchange

chromatography (MMC) have been investigated in order to provide retention and thus resolution of polar analytes. These separations can complement conventional RPLC analyses, or be combined into two-dimensional (2D) configurations (e.g., 2D-RPLC/HILIC), and provide an effective option for enhancing the separation power and coverage of the metabolome for complex matrices. However, innovations in column technologies/stationary phases have introduced increased options available for the chromatography of polar metabolites and the current state of the art for such analytes as applied to metabolic phenotyping is reviewed here.

2. LC/MS-based approaches

2.1. HILIC

HILIC is now well established as the LC mode most applied for separating polar metabolites [4,5] and enables the analysis of many polar metabolites that are difficult to analyse using RPLC. HILIC thus represents an attractive complementary technique to RPLC.

Whilst a wide range of HILIC stationary phases are available (including polymeric materials) silica and silica-based materials dominate this mode of separation. Numerous ligands can be bonded to silica to prepare new phases, depending on the properties of the analytes of interest; these modified phases are divided into four groups, neutral (amide, diol, cyano, etc.), positively charged (amino, imidazole, etc.), negatively charged (polyaspartic acid, bare silica, etc.) and zwitterionic materials (sulfoalkylbetaine or phosphorylcholine) [4–6]. The mobile phases commonly used in HILIC separations are based on an organic solvent (>90%), most often acetonitrile (ACN), and are modified with water, usually containing buffer salts (ammonium formate, ammonium acetate, 5–20 mM). Buffer salts are important as they modify the pH and ionic strength of the mobile phase thereby controlling analyte retention behavior and peak shape. The separation of analytes is usually via gradient elution, starting with a high percentage of organic solvent and followed by a gradual increase in the proportion of water (up to 40%). HILIC often requires long equilibration times after a gradient run but isocratic elution is suitable for analytes with a small polarity range. Sample diluent is also important for method performance and peak shape in HILIC. Overall, the sample diluent should contain as much of the weaker mobile phase solvent as possible, but the widespread use of ACN in this role may cause solubility issues.

Different HILIC materials, or combination of HILIC and RPLC separations, have been applied in metabolomics studies [7–9]. Method development has often included systematic efforts aimed at improving metabolome coverage, often using computational methods to probe the complex data that can be generated [10,11]. In general, the effective and fair comparison of chromatographic performance in the analysis of a large number of molecules of diverse physicochemical properties is not trivial and necessitates innovative approaches, especially for the generation of descriptive illustrations [10,11]. Such comparisons are performed either using MS/MS detection on triple quadrupole/Qtrap instruments [10,11] or using qTOF-MS in scanning mode [11,12]. Another perspective offered, and not yet fully explored, is the comparison of the content of biosamples e.g. cell culture vs blood plasma vs urine [11].

A typical targeted UHP-HILIC/MS application, performed on a silica-based BEH amide column, aimed at the determination of methylated amines and amino acids in human plasma/serum and urine in order to investigate the effects of age, sex, diet, body composition and physical activity is illustrated in Fig. 1 [13].

HILIC-MS has also been successfully applied to metabolically profile foodstuffs [14] and samples, such as fermentation broths [15]. In the last decade both targeted and untargeted methods have been used to analyze fruit, vegetables, meat, eggs, milk, honey, seafood, infant food, etc. for the determination of groups of metabolites such as nucleosides and nucleotides in baby foods [16] as well as sugars, organic acids and phenolic compounds in plant-based foods, such as vegetables, fruit, juices, and wine, etc. [17–20].

A newly introduced HILIC phase is a zwitterionic UHPLC column based on ethylene-bridged hybrid organic/inorganic (silica-based) particles bonded with sulfobetaine groups. When this phase was packed into columns treated to reduce analyte-metal interactions it showed benefits for the analysis of phosphorylated molecules in tissue extracts [21].

Although challenges remain HILIC is, as discussed further in **Section 3**, the most popular method for the analysis of polar metabolites and, when combined with RPLC, provides the simplest method for obtaining a moderately comprehensive metabolic profile of a sample.

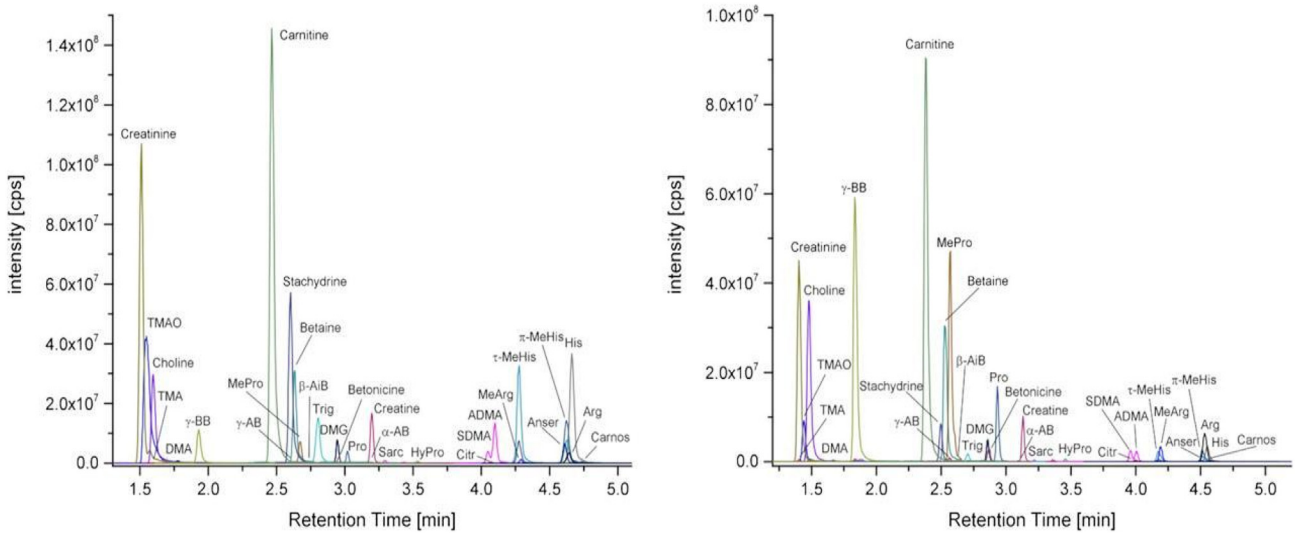

Fig. 1. HILIC-LC/MS of spiked urine (left) and blood plasma (right) samples. Peak identities: α-AB = α-aminobutyric acid; β-AiB = β-aminoisobutyric acid; γ-AB = γ-aminobutyric acid; γ-BB = γ-butyrobetaine; π-MeHis = π-methylhistidine; τ-MeHis = τ-methylhistidine; ADMA = asymmetric dimethylarginine; Anser = anserine; Arg = arginine; Carnos = carnosine; Citr = citrulline; DMA = dimethylamine; DMG = N,N-dimethylglycine; His = histidine; HyPro = hydroxyproline; MeArg = N-methylarginine; MePro = N-methylproline; Pro = proline; Sarc = sarcosine; SDMA = symmetric dimethylarginine; TMA = trimethylamine; TMAO = trimethylamine-N-oxide; Trig = trigonelline. Conditions: A: 1:1 mixture of ACN and 50 Mm AF, H_2O (pH 3.2), B: ACN with 0.05% FA. Gradient: 0–3 min, 12–51.5% A; 3–4 min 51.5% A; 4–5.9 min 51.5–85% A; 5.9–9 min 85-12% A. Column: HILIC BEH Amide (2.1 × 100 mm, 1.7 μm), Flow: 0.6 mL/min. MS type: QqQ. Reproduced from Ref. [13] with permission.

2.2. RPLC with derivatization

RPLC-MS is arguably the most widespread separation technique for metabolomic analysis, but clearly, its hydrophobic nature is not the most suitable choice for the separation of polar metabolites. Whilst adding salts, bases/acids to the mobile phase can improve polar metabolite retention [22,23] many polar/ionic metabolites remain poorly retained and a popular solution for this problem is to use derivatization to modify polar groups. Additional advantages of derivatization are higher *m/z* values, characteristic fragments and often superior ionization efficiency (increasing MS sensitivity) [24–26]. Amino acids have been popular targets for the LC-MS analysis of e.g., human plasma [27] or rat urine [28]. Other important classes, including short-chain fatty acids, bile acids and tryptophan metabolites can be derivatized with 3-nitrophenylhydrazine for UHPLC-MS/MS analysis [29]. Other recent applications of derivatization include the use of butanolysis-based derivatization of glycosaminoglycans [30] and dansylation for the analysis of phenolic or aliphatic hydroxylated compounds [31]. Derivatization has also been applied for neutral loss scans (NLS) of fragments associated with the derivatization reagent. An example is the use of diethyl ethoxymethylenemalonate (DEEMM) to modify amines (e.g., Ref. [32] to identify amino acids and other amines in an extract of *Carduus nutans* where the loss of 46 amu was diagnostic of a derivatized amine. Chromatograms obtained with and without derivatization are shown in Fig. 2.

Overall, pre-column derivatization is a useful tool for modifying the properties of metabolites that are difficult to retain by RPLC. Many varieties of derivatizing reagents are commercially available, specific for many functional groups, for use in metabolic phenotyping. However, derivatization, especially in biological matrices, whether pre or post-column, should be thoroughly studied and optimized. Derivatization may lead to quantification errors due to differences in reaction kinetics of the different metabolite classes, and be affected by the presence of multiple derivatizeable groups on the same analyte leading to several different dertivatives in variable amounts. The formation of unstable derivatives, with non-reproducible yields, must also be considered and investigated. However, in the minds of many analysts faced with the problems of profiling polar metabolites the advantages offered by RPLC with derivatization (improved retention, specificity and ionization, etc.), may compensate for all of these disadvantages.

2.3. Ionic interaction LC (IPC and IEX)

2.3.1. IPC

IPC is particularly useful for polar ionic metabolites e.g., nucleotides and phosphorylated sugars, particularly those that interact with metal surfaces in LC systems causing peak tailing and poor analysis. IPC enhances the separation of polar ionic compounds through the ion-pairing reagent (IPR) masking the ionizable group on the analyte, thereby facilitating hydrophobic interactions with the RPLC stationary phase. Volatile alkylamines, such as tributylamine (TBA) [33,34], hexylamine [35] and triethylamine (TEA), are the most common ion-pairing reagents and have been used with RP or porous graphitic carbon columns for the analysis of "problematic" polar acidic compounds (e.g., metabolites involved in central carbon metabolism, glycolysis, pentose phosphate pathways and the TCA cycle, etc).

For the analysis of positively charged metabolites, IPC methods using volatile perfluorinated acids have been employed [37,38], including nonafluoropentanoic acid (NFPA), pentafluoropropionic acid (PFPA), and heptafluorobutyric acid (HFBA) [36]. An example of this approach for the analysis of amino acids and related compounds in different matrices is a targeted IPC-MS method where HFBA was used in the quantification of 33 amino acids and biogenic amines in human urine for a study of tacrolimus nephrotoxicity [36], as presented in Fig. 3. Hexylamine has also been successfully used as an IPR for the analysis of coenzyme A esters, sugar nucleotides, and sugar bisphosphates [39].

However, whilst very useful chromatographically, IPC-MS suffers from the major limitation of system and source contamination, which effectively means that an instrument must be dedicated to its use/mode of ionization (+ve or -ve modes ESI) only. Also, ion suppression and adduct formation can adversely affect the usefulness of these methods and probably, for such reasons, IPC has had

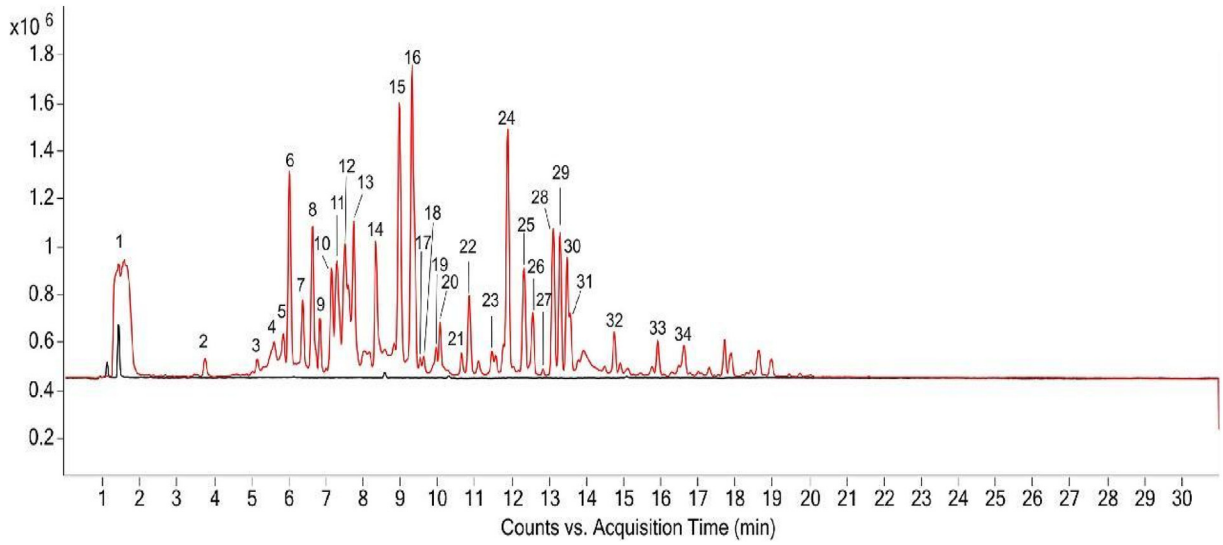

Fig. 2. RPLC-MS of extracts with (red) and without (black) derivatization with 1 – hydroxylamine derivative, 2 – histidine, 3 – *m/z* 282, 4 – arginine, 5 – *m/z* 365, 6 – asparagine, 7 – glutamine, 8 – serine, 9 – *m/z* 258.9, 10 – aspartic acid, 11 – *m/z* 232, 12 – *m/z* 188 (from blank), 13 – threonine, 14 – *m/z* 259.9, 15 – γ-aminobutyric acid, 16 – alanine, 17 – proline, 18 – *m/z* 274, 19 – *m/z* 288, 20 – tyrosine, 21 – *m/z* 274, 22 – *m/z* 324, 23 – *m/z* 242, 24 – valine, 25 – tyramine, 26 – tryptophan, 27 – ornithine, 28 – phenylalanine, 29 – isoleucine, 30 – leucine, 31 – lysine, 32 – putrescine, 33 – phenylethylamine, 34 – *m/z* 353. Only compounds identified against an authentic standard are listed. Conditions: A: H_2O, 0.1% FA., B: ACN. Gradient: 0–2 min, 10% B; 2–27 min, 10–100% B; 27–29 min, 100% B; 29–31 min, 100–10% B; Column: Zorbax Eclipse Plus C18 (3.0 × 100 mm, 1.8 μm). Flow: 0.50 mL/min. MS type: QqQ. Reproduced from Ref. [32] with permission.

limited take-up by the metabolomics community.

2.3.2. IEX/IC

Ion-exchange chromatography (IEX) or ion-chromatography (IC) represents another alternative for the separation of polar ionic metabolites. Retention in IEX is based on ionic interactions and adsorption and is well suited to hydrophilic polar molecules whilst any solubility issues are minimized by the aqueous mobile phases used. IEX stationary phases include both polymeric and silica-based materials, with the former being stable over a wide pH range [40].

IEX-MS has been applied for global profiling or quantification of metabolites involved in pathways such as glycolysis, Calvin cycle, plant cell wall biosynthesis, and the TCA cycle [41–44]. Capillary IEX has also been coupled to HRMS for enhanced sensitivity in metabolomics [43]. The ability of IEX to separate isomeric species, such as glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate; citric and isocitric acid; *cis*-aconitate and *trans*-aconitate; fructose-1,6-diphosphate and fructose-2,6-diphosphate, which are unresolved by HILIC, is illustrated in Fig. 4.

The limitations of IEX are that the ionic interactions that govern the analyte retention can require high-concentration buffers for analyte elution and this is not easily compatible with ESI. Additionally, IEX provides relatively poor kinetic performance (low peak capacity can be attained compared to RPLC). It is perhaps for these reasons that, like IPC, IEX/IC applications in metabolomics have been limited.

2.4. Mixed-mode and 2D-LC

2.4.1. MMC

Mixed-mode, or multimode chromatography (MMC), offers the ability to separate a wide range of metabolites, including polar and charged molecules in a single analysis. MMC improves separation power by effectively using different approaches, such as the connection of two columns with different separation modes, the mixing of two stationary phases in a column or the use of a single mixed-mode stationary phase based on the chemical synthesis of new stationary phases with different functional groups. MMC can be performed on RP/anion-exchange (AX), RP/cation-exchange (CX), HILIC/AX, and HILIC/CX bimodal phases, as well as RP/AX/CX and HILIC/AX/CX trimodal materials columns thereby retaining both hydrophobic metabolites via RP and ionized analytes by ion-exchange mechanisms. Depending on mobile phase pH, the functional groups in mixed-mode columns and ionic analytes may be ionized or not [45]. Ion-exchangers are classified as strong or weak anion or cation exchangers (SAX, SCX, WAX, SCX, respectively) and RPLC, RPLC/IEX has the advantage over RPLC of providing separations for both non- and highly polar compounds [46]. Thus, a recent comparison of mixed-mode RP/IEC versus RPLC showed increased retention of highly polar sarin-related metabolites by MMC [47] and recently an RP-AX separation on a CSH Phenyl-Hexyl column was used to analyze tricarboxylic acid cycle metabolites, etc., including even isobaric compounds, such as isocitric acid and citric acid, as well as malic and fumaric acids, without either ion-pairing reagents or other mobile phase additives (Fig. 5) [48]. The column was modified with a hybrid organic-inorganic surface that reduced analyte-metal interactions, improving both MS data quality and increasing analyte recovery, particularly for phosphorylated metabolites.

Combining HILIC partition with IEX mechanisms, mixed-mode stationary phases with a long-alkyl chain ligand and a hydrophilic polar terminal ion-exchange group (bonded on the silica gel support), has resulted in improved separation selectivity for many polar compounds. For example, HILIC/IEC showed improved peak shape and resolution compared to previously reported HILIC methods for 23 underivatized amino acids [49].

A single mixed-mode column, or two columns connected in series, will generally achieve similar performance to two single-mode columns whilst reducing solvent waste, sample and material consumption. As an example, an in-line two-column IEX-RPLC approach has been developed for the simultaneous determination of amino acids, acylcarnitines, and organic acids in human plasma and urine [50]. This approach also combined ionization in both

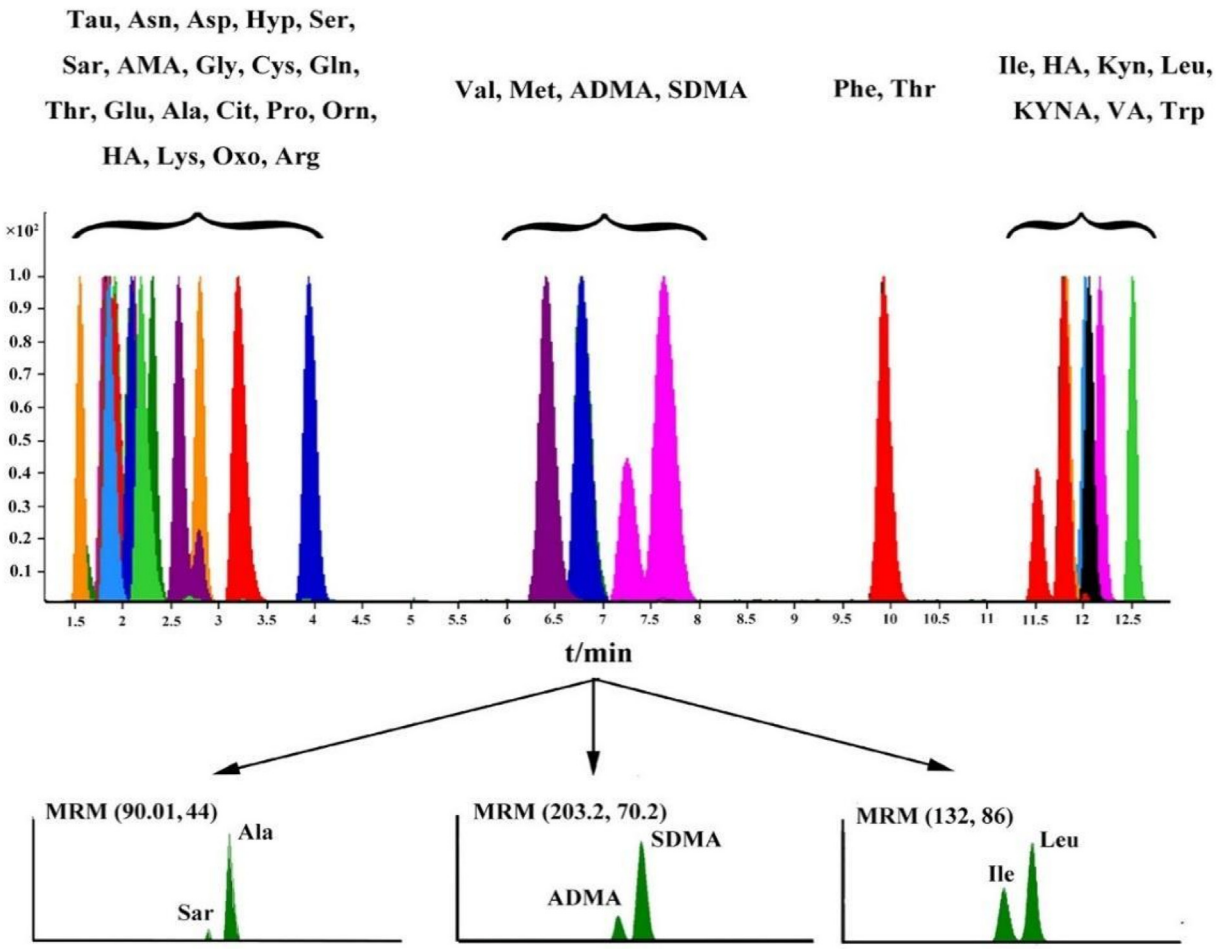

Fig. 3. Ion chromatogram showing MRM transitions on 33 amino acids and biogenic amines in a 13 min IPC-MS/MS run. Conditions: A: H_2O 0.2% FA and 0.02% HFBA. B: MeOH. Gradient: 0–6 min, 2–5%; B, 6–9 min, 5–20% B; 9–12 min, 20–80% B; 12–13 min, 80–100% B. Column: Zorbax SB-C18 column (3.0 × 150 mm, 5 μm). Flow: 0.4 mL/min. Reproduced from Ref. [36] with permission.

polarities in MS. Furthermore, newly developed mixed-mode columns can also be used for both RP-type separations and HILIC for neutral polar molecules [51]. These RP/ZWIX phases have obvious potential application areas for polar metabolite analysis. Also, recently, a unified-HILIC/AEX/MS method that provided greater coverage for polar metabolite analysis than conventional HILIC/MS methods, in both targeted and nontargeted metabolomics, has been described [52].

In addition to biofluids, MMC/MS has also applications in plant/food analysis including the analysis of analytes, such as glycerophospholipids in tobacco [53].

Mixed-mode phases clearly offer flexibility in retention and selectivity adjustment and provide interesting new possibilities for the simultaneous LC/MS analysis of polar endogenous metabolites.

2.4.2. 2D-LC

2D-LC often offers increased peak capacity and metabolome coverage compared to single-column analysis [54]. 2D-LC can either a) be based on a simple single heart-cut (LC-LC) or 2) be fully comprehensive (LC × LC). The major difference between these modes is the process by which fractions from the first dimension (1D) eluent are transferred to the second dimension (2D) column. Whilst offering improved separations 2D-LC can be technically demanding, as the development and optimization of such methods involves finding the optimal and the most compatible conditions between the two dimensions. Thus, the optimal mobile phase for one dimension limits compatibility with that of the other.

Applications of 2D-LC in metabolomics have been made to targeted, or pseudo-targeted, analyses taking advantage of improved LC resolution [55]. For untargeted analysis data treatment (mainly peak alignment) remain challenging and although 2D-LC for metabolomics was described over a decade ago [56] its use remains limited to a few expert groups engaged in biological [57–60] or plant/food [61–64] applications. 2D-LC/MS for metabolomics has been comprehensively reviewed [65] but needs further technological improvements before finding wider metabolic applications.

3. Current Trends in Polar Metabolome Analysis by LC/MS

LC-MS-based metabolomics has been employed for over 2 decades and applications are still increasing. In Fig. 6 the trends for publications on the polar metabolome using HILIC, ion pair and derivatization with RPLC are shown. The search, performed in Scopus (April 2022), used these terms connected with AND metabolomics AND LC-MS in the Abstract, Title and Keywords. Manual inspection was subsequently performed to remove irrelevant works e.g., removing those describing GC-MS-derivatization but with LC in the abstract and thus erroneously identified by Scopus.

This plot is not a systematic review and we do not claim that it

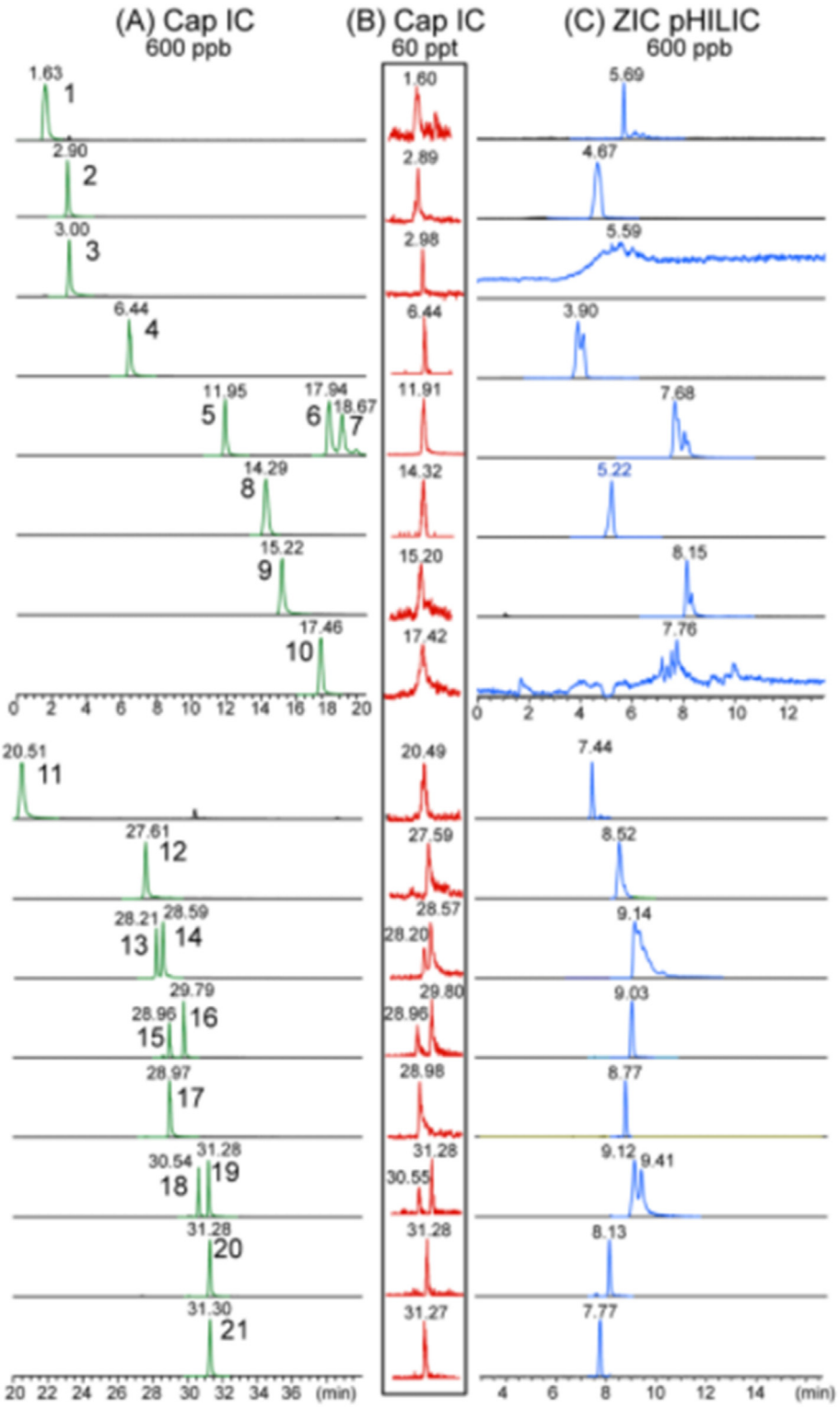

Fig. 4. Separation of 21 metabolites 600 ppb by Cap IC (A), 60 ppt by Cap IC (B), and 600 ppb by HILIC (C). Injection volume of 5 μL. Standard mixture for IC was diluted in H_2O and for HILIC was diluted in ACN/H_2O (3:1, v/v). Key: 1 D-glucose, 2 mevalonate, 3 lactate, 4 uridine, 5 α-D-glucose 1-phosphate, 6 α-D-glucose 6-phosphate, 7 D-fructose 6-phosphate, 8 adenosine 3′-5′-cyclic monophosphate (cAMP), 9 tartrate, 10 2-oxoglutarate, 11 adenosine 5′-monophosphate (AMP), 12 2-phosphoglycerate, 13 citrate, 14 isocitrate, 15 *cis*-aconitate, 16 *trans*-aconitate, 17 phosphoenolpyruvate, 18 D-fructose-1,6-diphosphate, 19 D-fructose-2,6-diphosphate, 20 dihydroxy acetone-phosphate, 21 inosine 5′-monophosphate (IMP). Reproduced from Ref. [43] with permission.

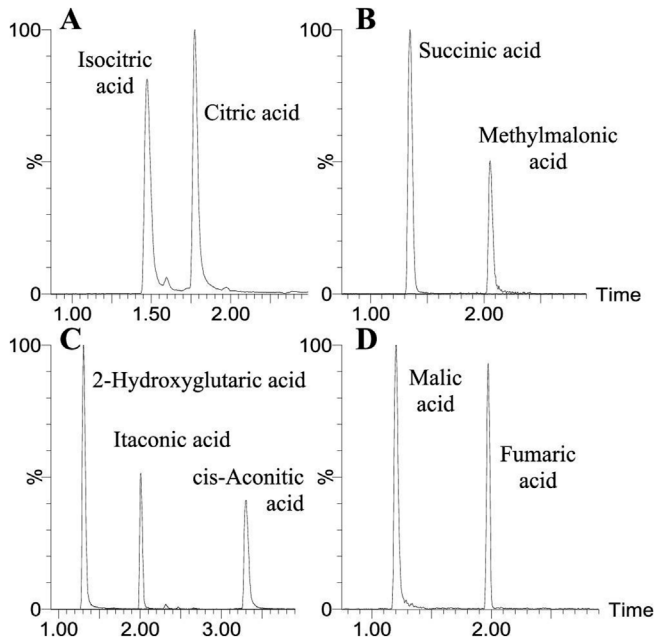

Fig. 5. A mixed mode separation showing the resolution of critical pairs of polar organic acids. Isocitric and citric acids (A), methylmalonic and succinic acids (B), itaconic, 2-hydroxyglutaric, and *cis*-aconitic acids (C), malic and fumaric acids (D). Conditions: A: H_2O, 0.1% FA., B: ACN, 0.1% F.A. Gradient: 0–4.0 min, 0–25% B; 7.0–8.0 min, 95% B; 8.0–10.0 min, 0% B. Column: CSH Phenyl-Hexyl (2.1 × 100 mm, 1.7 μm). Flow: 0.40 mL/min. Reproduced from Ref. [48] with permission.

provides the absolute numbers of publications (so, possibly publications were not found even though they used e.g., HILIC-MS but did not report this in the title/abstract/keywords, etc., but only in the full text or the supplementary information). However, Fig. 6 provides a useful "snapshot" of the evolving trends for polar metabolome analysis and shows the relative importance of the major approaches currently used. Importantly, it is evident that the numbers are increasing for all approaches with an almost tenfold increase in publications since 2008. Also, it is evident that whilst HILIC continues to be the major platform for this, derivatization is still popular for metabolome analysis. Other chromatographic approaches e.g., ANP and IEC, are less widely applicable than HILIC for high throughput metabolomic studies due to compatibility issues with MS and ESI, caused by the solvents and additives used.

The adoption of better resolution separation methods is a crucial area for future improvement in polar metabolome profiling. Capillary (Cap) LC and the recent advances in UPLC and UHPLC stationary phases and columns technology discussed above are increasingly widely adopted alternatives to conventional HPLC that offer superior resolution. Along with this strategy of using columns providing greater chromatographic efficieny, methods using so-called "2-dimensional" LC separations, offer a way to broaden the coverage of the polar metabolome [66]. Current trends in liquid chromatography also include nano-LC columns that provide enhanced sensitivity and significantly reduced solvent consumption. Particularly for the study of extremely polar compounds, new column technologies, such as specifically treated phases and materials (HPLC and UHPLC), are regularly being introduced offering increased chromatographic efficiency and selectivity. Recent applications include HILIC-based microbore columns (1.0 mm i.d.), with rapid separations (3.3 min) and high mobile phase linear velocities, in combination with high-resolution mass spectrometers for high-throughput endogenous metabolic profiling of polar metabolites [67]. In addition, innovative materials featuring microchips, particularly pillar array columns, are attracting interest for in-field or real-time analysis (although as yet there are no published applications of these emerging technologies for metabolomics).

Another promising alternative technique for polar metabolome analysis, which, although not the focus of this review merits mention is supercritical fluid chromatography (SFC). With the availability of a new generation of equipment, this approach has once more attracted attention (reviewed in Ref. [68]. SFC has also benefited from advancements in column chemistry (including the

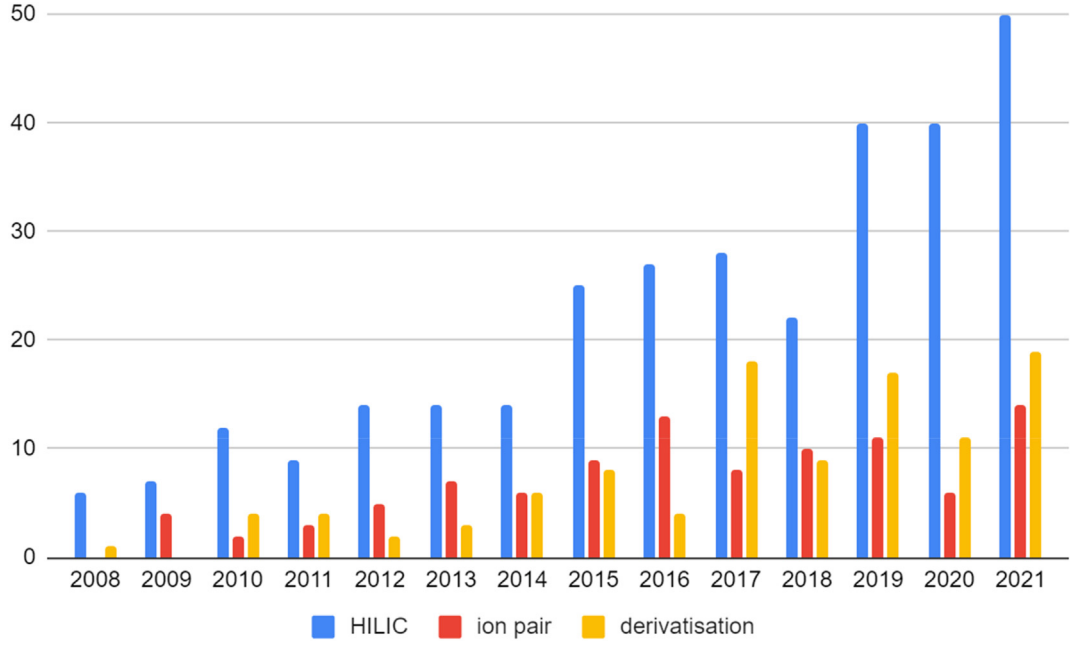

Fig. 6. Plot of publications/year found using the search terms "metabolomics" AND "LC-MS" AND ("HILIC" OR "ion pair" OR "derivatization"). Search performed in Scopus (April 2022) using Abstract, Title and Keywords.

use of sub-2 m particles) specifically designed for SFC and applicable to polar metabolme analysis [69]. This has allowed the analysis of a wide variety of polar metabolites including carbohydrates, amino acids and nucleosides [68]. For a recent commentary on the current state of the art in SFC and its future prospect see the recent review by Si-Hung and Bamba [70].

Of couse methods for analysing the polar metabolome face the same general problems as the rest of the field with data analysis proving especially challenging by both targeted and untargeted profiling. Similarly, attempting to merge data acquired with various experimental configurations (e.g. HILIC−MS and RPLC−MS; APCI-MS and ESI-MS) is incredibly difficult, if not downright impractical. Methods for this task still need to be developed but require high-quality data from consistent sample sets and carefully thought-out trials [71]. However this is done, the task of metabolite identification (MetID) is still a major challenge in LC-MS-based metabolomics studies. The most promising approaches for MetID will require a combination of high-resolution MS (HRMS), MS/MS analysis, probably with ion mobility to provide improved spectral quality and collision cross section (CCS) data and contemporary bioinformatics technologies [72]. In this aspect the study and generation of spectral libraries via the LC-MS of reference standards is of utmost importance and is, in the authors opinion, worth the effort needed [73]. Last but not least, sample preparation may prove critical and necessitate investigation, especially in targeted modes, for certain analytes, either due to their polarity, which may limit applicability of extraction methods and materials [74], or due to analyte instability [75].

4. Conclusions

The analysis of the polar metabolome using LC-MS-based methods represents one of several major challenges for metabolic phenotyping. HILIC represents a good basic platform for many types of polar metabolites and, although it is not a panacea, in the medium term it will remain the first choice for these analytes. Other methods show promise and offer complementary views of the metabolome for analytes that are not amenable to RPLC or HILIC. Derivatization remains an important approach, particularly for targeted metabolomics, and it is likely that this will continue. IPC is also a useful approach but in practice requires an LC/MS system to be dedicated to its use, in a single ESI polarity. The analysis of the polar metabolome is becoming an increasing focus for researchers engaged in metabolic phenotyping and we also see increased awareness of this need interest from column manufacturers through the development of mixed-mode approaches that may address some of the issues hindering this segment of metabolic profiling.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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References

[1] A. Lioupi, M. Marinaki, C. Virgiliou, H. Gika, I. Wilson, G. Theodoridis, State-of-the-art in LC−MS Approaches for Probing the Polar Metabolome, 2021. https://doi.org/10.1039/9781839163524-00001.
[2] P. Yin, G. Xu, Current state-of-the-art of nontargeted metabolomics based on liquid chromatography−mass spectrometry with special emphasis in clinical applications, J. Chromatogr., A 1374 (2014) 1−13. https://doi.org/10.1016/j.chroma.2014.11.050.
[3] H.G. Gika, G.A. Theodoridis, R.S. Plumb, I.D. Wilson, Current practice of liquid chromatography−mass spectrometry in metabolomics and metabonomics, J. Pharm. Biomed. Anal. 87 (2014) 12−25. https://doi.org/10.1016/j.jpba.2013.06.032.
[4] Q. Hu, H. Tang, Y. Wang, Challenges in analysis of hydrophilic metabolites using chromatography coupled with mass spectrometry, J. Anal. Test. 4 (2020) 140−162. https://doi.org/10.1007/s41664-020-00126-z.
[5] D.-Q. Tang, L. Zou, X.-X. Yin, C.N. Ong, HILIC-MS for metabolomics: an attractive and complementary approach to RPLC-MS, Mass Spectrom. Rev. 35 (2016) 574−600. https://doi.org/10.1002/mas.21445.
[6] G. Qing, J. Yan, X. He, X. Li, X. Liang, Recent advances in hydrophilic interaction liquid interaction chromatography materials for glycopeptide enrichment and glycan separation, TrAC, Trends Anal. Chem. 124 (2020), 115570. https://doi.org/10.1016/j.trac.2019.06.020.
[7] C. Lavarello, S. Barco, M. Bartolucci, I. Panfoli, E. Magi, G. Tripodi, A. Petretto, G. Cangemi, Development of an accurate mass retention time database for untargeted metabolomic analysis and its application to plasma and urine pediatric samples, Molecules 26 (2021) 4256. https://doi.org/10.3390/molecules26144256.
[8] E. Iturrospe, K.M. Da Silva, B. Talavera Andújar, M. Cuykx, J. Boeckmans, T. Vanhaecke, A. Covaci, A.L.N. van Nuijs, An exploratory approach for an oriented development of an untargeted hydrophilic interaction liquid chromatography-mass spectrometry platform for polar metabolites in biological matrices, J. Chromatogr., A 1637 (2021), 461807. https://doi.org/10.1016/j.chroma.2020.461807.
[9] J.H. Kim, Q. Yan, K. Uppal, X. Cui, C. Ling, D.I. Walker, J.E. Heck, O.S. von Ehrenstein, D.P. Jones, B. Ritz, Metabolomics analysis of maternal serum exposed to high air pollution during pregnancy and risk of autism spectrum disorder in offspring, Environ. Res. 196 (2021), 110823. https://doi.org/10.1016/j.envres.2021.110823.
[10] I. Sampsonidis, M. Witting, W. Koch, C. Virgiliou, H.G. Gika, P. Schmitt-Kopplin, G.A. Theodoridis, Computational analysis and ratiometric comparison approaches aimed to assist column selection in hydrophilic interaction liquid chromatography−tandem mass spectrometry targeted metabolomics, J. Chromatogr., A 1406 (2015) 145−155. https://doi.org/10.1016/j.chroma.2015.06.008.
[11] K. Serafimov, M. Lämmerhofer, Metabolic profiling workflow for cell extracts by targeted hydrophilic interaction liquid chromatography-tandem mass spectrometry, J. Chromatogr., A 1684 (2022), 463556. https://doi.org/10.1016/j.chroma.2022.463556.
[12] F. Hosseinkhani, L. Huang, A.-C. Dubbelman, F. Guled, A.C. Harms, T. Hankemeier, Systematic evaluation of HILIC stationary phases for global metabolomics of human plasma, Metabolites 12 (2022) 165. https://doi.org/10.3390/metabo12020165.
[13] T. Roggensack, B. Merz, N. Dick, A. Bub, R. Krüger, Targeted ultra-performance liquid chromatography/tandem mass spectrometric quantification of methylated amines and selected amino acids in biofluids, Rapid Commun. Mass Spectrom. 34 (2020), e8646. https://doi.org/10.1002/rcm.8646.
[14] G. Marrubini, P. Appelblad, M. Maietta, A. Papetti, Hydrophilic interaction chromatography in food matrices analysis: an updated review, Food Chem. 257 (2018) 53−66. https://doi.org/10.1016/j.foodchem.2018.03.008.
[15] S. Schiesel, M. Lämmerhofer, W. Lindner, Multitarget quantitative metabolic profiling of hydrophilic metabolites in fermentation broths of β-lactam antibiotics production by HILIC−ESI−MS/MS, Anal. Bioanal. Chem. 396 (2010) 1655−1679. https://doi.org/10.1007/s00216-009-3432-2.
[16] M. Mateos-Vivas, E. Rodríguez-Gonzalo, J. Domínguez-Álvarez, D. García-Gómez, R. Carabias-Martínez, Determination of nucleosides and nucleotides in baby foods by hydrophilic interaction chromatography coupled to tandem mass spectrometry in the presence of hydrophilic ion-pairing reagents, Food Chem. 211 (2016) 827−835. https://doi.org/10.1016/j.foodchem.2016.05.091.
[17] T. Doco, P. Williams, E. Meudec, V. Cheynier, N. Sommerer, Complex carbohydrates of red wine: characterization of the extreme diversity of neutral oligosaccharides by ESI-MS, J. Agric. Food Chem. 63 (2015) 671−682. https://doi.org/10.1021/jf504795g.
[18] A. Sentkowska, M. Biesaga, K. Pyrzynska, Effects of the operation parameters on HILIC separation of flavonoids on zwitterionic column, Talanta 115 (2013) 284−290. https://doi.org/10.1016/j.talanta.2013.05.005.
[19] P. Kubica, J. Namieśnik, A. Wasik, Comparison of hydrophilic interaction and reversed phase liquid chromatography coupled with tandem mass spectrometry for the determination of eight artificial sweeteners and common

steviol glycosides in popular beverages, J. Pharm. Biomed. Anal. 127 (2016) 184–192. https://doi.org/10.1016/j.jpba.2016.01.006.
[20] C. Virgiliou, D. Kanelis, A. Pina, H. Gika, C. Tananaki, A. Zotou, G. Theodoridis, A targeted approach for studying the effect of sugar bee feeding on the metabolic profile of Royal Jelly, J. Chromatogr., A 1616 (2020), 460783. https://doi.org/10.1016/j.chroma.2019.460783.
[21] A. Lioupi, C. Virgiliou, T.H. Walter, K.M. Smith, P. Rainville, I.D. Wilson, G. Theodoridis, H.G. Gika, Application of a hybrid zwitterionic hydrophilic interaction liquid chromatography column in metabolic profiling studies, J. Chromatogr., A 1672 (2022), 463013. https://doi.org/10.1016/j.chroma.2022.463013.
[22] Q. Hu, H. Tang, Y. Wang, Challenges in analysis of hydrophilic metabolites using chromatography coupled with mass spectrometry, J. Anal. Test. 4 (2020) 140–162. https://doi.org/10.1007/s41664-020-00126-z.
[23] S. Zhao, L. Li, Chemical derivatization in LC-MS-based metabolomics study, TrAC, Trends Anal. Chem. 131 (2020), 115988. https://doi.org/10.1016/j.trac.2020.115988.
[24] J.P. Violi, D.P. Bishop, M.P. Padula, J.R. Steele, K.J. Rodgers, Considerations for amino acid analysis by liquid chromatography-tandem mass spectrometry: a tutorial review, TrAC, Trends Anal. Chem. 131 (2020), 116018. https://doi.org/10.1016/j.trac.2020.116018.
[25] C.C. Hughes, Chemical labeling strategies for small molecule natural product detection and isolation, Nat. Prod. Rep. 38 (2021) 1684–1705. https://doi.org/10.1039/D0NP00034E.
[26] C. Calderón, M. Lämmerhofer, Enantioselective metabolomics by liquid chromatography-mass spectrometry, J. Pharm. Biomed. Anal. 207 (2022), 114430. https://doi.org/10.1016/j.jpba.2021.114430.
[27] C. Bruno, C. Veyrat-Durebex, C.H. Lumbu Lukuntonda, C.R. Andres, C. Moreau, C. Bendavid, C. Homedan, F. Labarthe, M. Tardieu, A. Bigot, F. Maillot, I. Benzde Bretagne, H. Blasco, Validation of plasma amino acid profile using UHPLC-mass spectrometer (QDa) as a screening method in a metabolic disorders reference centre: performance and accreditation concerns, Clin. Biochem. 92 (2021) 34–45. https://doi.org/10.1016/j.clinbiochem.2021.03.004.
[28] N. Gray, R.S. Plumb, I.D. Wilson, J.K. Nicholson, A validated UPLC-MS/MS assay for the quantification of amino acids and biogenic amines in rat urine, J. Chromatogr. B 1106–1107 (2019) 50–57. https://doi.org/10.1016/j.jchromb.2018.12.028.
[29] H.-Y. Liao, C.-Y. Wang, C.-H. Lee, H.-L. Kao, W.-K. Wu, C.-H. Kuo, Development of an efficient and sensitive chemical derivatization-based LC–MS/MS method for quantifying gut microbiota-derived metabolites in human plasma and its application in studying cardiovascular disease, J. Proteome Res. 20 (2021) 3508–3518. https://doi.org/10.1021/acs.jproteome.1c00147.
[30] K. Matyjaszczyk-Gwarda, A. Kij, M. Olkowicz, B. Fels, K. Kusche-Vihrog, M. Walczak, S. Chlopicki, Simultaneous quantification of selected glycosaminoglycans by butanolysis-based derivatization and LC-SRM/MS analysis for assessing glycocalyx disruption in vitro and in vivo, Talanta 238 (2022), 123008. https://doi.org/10.1016/j.talanta.2021.123008.
[31] S. Zhao, X. Luo, L. Li, Chemical isotope labeling LC-MS for high coverage and quantitative profiling of the hydroxyl submetabolome in metabolomics, Anal. Chem. 88 (2016) 10617–10623. https://doi.org/10.1021/acs.analchem.6b02967.
[32] L.S. Maciel, A. Marengo, P. Rubiolo, I. Leito, K. Herodes, Derivatization-targeted analysis of amino compounds in plant extracts in neutral loss acquisition mode by liquid chromatography-tandem mass spectrometry, J. Chromatogr., A 1656 (2021), 462555. https://doi.org/10.1016/j.chroma.2021.462555.
[33] F. Michopoulos, N. Whalley, G. Theodoridis, I.D. Wilson, T.P.J. Dunkley, S.E. Critchlow, Targeted profiling of polar intracellular metabolites using ion-pair-high performance liquid chromatography and -ultra high performance liquid chromatography coupled to tandem mass spectrometry: applications to serum, urine and tissue extracts, J. Chromatogr., A 1349 (2014) 60–68. https://doi.org/10.1016/j.chroma.2014.05.019.
[34] P. Kiefer, N. Delmotte, J.A. Vorholt, Nanoscale ion-pair reversed-phase HPLC–MS for sensitive metabolome analysis, Anal. Chem. 83 (2011) 850–855. https://doi.org/10.1021/ac102445r.
[35] L. Coulier, R. Bas, S. Jespersen, E. Verheij, M.J. van der Werf, T. Hankemeier, Simultaneous quantitative analysis of metabolites using ion-pair liquid Chromatography–Electrospray ionization mass spectrometry, Anal. Chem. 78 (2006) 6573–6582. https://doi.org/10.1021/ac0607616.
[36] T. Xia, S. Fu, Q. Wang, Y. Wen, S. Chan, S. Zhu, S. Gao, X. Tao, F. Zhang, W. Chen, Targeted metabolomic analysis of 33 amino acids and biogenic amines in human urine by ion-pairing HPLC-MS/MS: biomarkers for tacrolimus nephrotoxicity after renal transplantation, Biomed, Chromatography 32 (2018) e4198. https://doi.org/10.1002/bmc.4198.
[37] M. Armstrong, K. Jonscher, N.A. Reisdorph, Analysis of 25 underivatized amino acids in human plasma using ion-pairing reversed-phase liquid chromatography/time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. RCM. 21 (2007) 2717–2726. https://doi.org/10.1002/rcm.3124.
[38] X. Chen, H. Wu, Y. Cao, X. Yao, L. Zhao, T. Wang, Y. Yang, D. Lv, Y. Chai, Y. Cao, Z. Zhu, Ion-pairing chromatography on a porous graphitic carbon column coupled with time-of-flight mass spectrometry for targeted and untargeted profiling of amino acid biomarkers involved in Candida albicans biofilm formation, Mol. Biosyst. 10 (2013) 74–85. https://doi.org/10.1039/C3MB70240E.
[39] L. Coulier, R. Bas, S. Jespersen, E. Verheij, M.J. van der Werf, T. Hankemeier, Simultaneous quantitative analysis of metabolites using ion-pair liquid Chromatography–Electrospray ionization mass spectrometry, Anal. Chem. 78 (2006) 6573–6582. https://doi.org/10.1021/ac0607616.
[40] C. Bruggink, D. Jensen, Combining ion chromatography with mass spectrometry and inductively coupled plasma-mass spectrometry: annual review 2020, Anal. Sci. Adv. 2 (2021) 238–249. https://doi.org/10.1002/ansa.202000120.
[41] H.F.N. Kvitvang, K.A. Kristiansen, P. Bruheim, Assessment of capillary anion exchange ion chromatography tandem mass spectrometry for the quantitative profiling of the phosphometabolome and organic acids in biological extracts, J. Chromatogr., A 1370 (2014) 70–79. https://doi.org/10.1016/j.chroma.2014.10.029.
[42] K. Burgess, D. Creek, P. Dewsbury, K. Cook, M.P. Barrett, Semi-targeted analysis of metabolites using capillary-flow ion chromatography coupled to high-resolution mass spectrometry, Rapid Commun. Mass Spectrom. RCM. 25 (2011) 3447–3452. https://doi.org/10.1002/rcm.5247.
[43] J. Wang, T.T. Christison, K. Misuno, L. Lopez, A.F. Huhmer, Y. Huang, S. Hu, Metabolomic profiling of anionic metabolites in head and neck cancer cells by capillary ion chromatography with orbitrap mass spectrometry, Anal. Chem. 86 (2014) 5116–5124. https://doi.org/10.1021/ac500951v.
[44] A. Gomez-Gomez, J. Marcos, P. Aguilera, J. To-Figueras, O.J. Pozo, Comprehensive analysis of the tryptophan metabolome in urine of patients with acute intermittent porphyria, J. Chromatogr. B 1060 (2017) 347–354. https://doi.org/10.1016/j.jchromb.2017.06.030.
[45] A.P. Vilches, S.H. Norström, D. Bylund, Direct analysis of free amino acids by mixed-mode chromatography with tandem mass spectrometry, J. Separ. Sci. 40 (2017) 1482–1492. https://doi.org/10.1002/jssc.201601097.
[46] L. Wang, W. Wei, Z. Xia, X. Jie, Z.Z. Xia, Recent advances in materials for stationary phases of mixed-mode high-performance liquid chromatography, TrAC, Trends Anal. Chem. 80 (2016) 495–506. https://doi.org/10.1016/j.trac.2016.04.001.
[47] M.F. Vokuev, T.M. Baygildiev, I.V. Plyushchenko, Y.A. Ikhalaynen, R.L. Ogorodnikov, I.K. Solontsov, A.V. Braun, E.I. Savelieva, I.V. Rybalchenko, I.A. Rodin, Untargeted and targeted analysis of sarin poisoning biomarkers in rat urine by liquid chromatography and tandem mass spectrometry, Anal. Bioanal. Chem. 413 (2021) 6973–6985. https://doi.org/10.1007/s00216-021-03655-3.
[48] K.M. Smith, I.D. Wilson, P.D. Rainville, Sensitive and reproducible mass spectrometry-compatible RP-UHPLC analysis of tricarboxylic acid cycle and related metabolites in biological fluids: application to human urine, Anal. Chem. 93 (2021) 1009–1015. https://doi.org/10.1021/acs.analchem.0c03863.
[49] M.S. Choi, S.U. Rehman, I.S. Kim, H.-J. Park, M.-Y. Song, H.H. Yoo, Development of a mixed-mode chromatography with tandem mass spectrometry method for the quantitative analysis of 23 underivatized amino acids in human serum, J. Pharm. Biomed. Anal. 145 (2017) 52–58. https://doi.org/10.1016/j.jpba.2017.06.040.
[50] A. Le, J. Mak, T.M. Cowan, Metabolic profiling by reversed-phase/ion-exchange mass spectrometry, J. Chromatogr. B 1143 (2020), 122072. https://doi.org/10.1016/j.jchromb.2020.122072.
[51] M. Ferri, S. Bäurer, A. Carotti, M. Wolter, B. Alshaar, J. Theiner, T. Ikegami, C. West, M. Lämmerhofer, Fragment-based design of zwitterionic, strong cation- and weak anion-exchange type mixed-mode liquid chromatography ligands and their chromatographic exploration, J. Chromatogr., A 1621 (2020), 461075. https://doi.org/10.1016/j.chroma.2020.461075.
[52] K. Nakatani, Y. Izumi, M. Takahashi, T. Bamba, Unified-hydrophilic-interaction/anion-exchange liquid chromatography mass spectrometry (Unified-HILIC/AEX/MS): a single-run method for comprehensive and simultaneous analysis of polar metabolome, Anal. Chem. 94 (2022) 16877–16886. https://doi.org/10.1021/acs.analchem.2c03986.
[53] S. Flor, L. Sosa Alderete, C. Dobrecky, V. Tripodi, E. Agostini, S. Lucangioli, LC-ESI-MS/MS method for the profiling of glycerophospholipids and its application to the analysis of tobacco hairy roots as early indicators of phenol pollution, Chromatographia 84 (2021) 597–608. https://doi.org/10.1007/s10337-021-04034-x.
[54] M. Grübner, A. Dunkel, F. Steiner, T. Hofmann, Systematic evaluation of liquid chromatography (LC) column combinations for application in two-dimensional LC metabolomic studies, Anal. Chem. 93 (2021) 12565–12573. https://doi.org/10.1021/acs.analchem.1c01857.
[55] W. Lv, L. Wang, Q. Xuan, X. Zhao, X. Liu, X. Shi, G. Xu, Pseudotargeted method based on parallel column two-dimensional liquid chromatography-mass spectrometry for broad coverage of metabolome and lipidome, Anal. Chem. 92 (2020) 6043–6050. https://doi.org/10.1021/acs.analchem.0c00372.
[56] Y. Wang, R. Lehmann, X. Lu, X. Zhao, G. Xu, Novel, fully automatic hydrophilic interaction/reversed-phase column-switching high-performance liquid chromatographic system for the complementary analysis of polar and apolar compounds in complex samples, J. Chromatogr., A 1204 (2008) 28–34. https://doi.org/10.1016/j.chroma.2008.07.010.
[57] J. Feng, Q. Zhong, J. Kuang, J. Liu, T. Huang, T. Zhou, Simultaneous analysis of the metabolome and lipidome using polarity partition two-dimensional liquid chromatography–mass spectrometry, Anal. Chem. 93 (2021) 15192–15199. https://doi.org/10.1021/acs.analchem.1c03905.
[58] H. Li, W. Wei, Z. Li, M. Wang, X. Wei, M. Cheng, C. Yao, Q. Bi, J. Zhang, J. Li, D. Guo, An enhanced strategy integrating offline two-dimensional separation with data independent acquisition mode and deconvolution: Characterization of metabolites of Uncaria rhynchophylla in rat plasma as a case, J. Chromatogr. B 1181 (2021), 122917. https://doi.org/10.1016/j.jchromb.2021.122917.
[59] J. Xu, S. Zheng, M. Li, X. Liu, H. Sun, Z. Guo, J. Wei, L. Jia, W. Sun,

A comprehensive 2D-LC/MS/MS profile of the normal human urinary metabolome, Diagnostics 12 (2022) 2184. https://doi.org/10.3390/diagnostics12092184.
[60] S. Wang, L. Zhou, Z. Wang, X. Shi, G. Xu, Simultaneous metabolomics and lipidomics analysis based on novel heart-cutting two-dimensional liquid chromatography-mass spectrometry, Anal. Chim. Acta 966 (2017) 34–40. https://doi.org/10.1016/j.aca.2017.03.004.
[61] L. Montero, E. Ibáñez, M. Russo, R. di Sanzo, L. Rastrelli, A.L. Piccinelli, R. Celano, A. Cifuentes, M. Herrero, Metabolite profiling of licorice (Glycyrrhiza glabra) from different locations using comprehensive two-dimensional liquid chromatography coupled to diode array and tandem mass spectrometry detection, Anal. Chim. Acta 913 (2016) 145–159. https://doi.org/10.1016/j.aca.2016.01.040.
[62] M. Krauze-Baranowska, D. Głód, M. Kula, M. Majdan, R. Hałasa, A. Matkowski, W. Kozłowska, A. Kawiak, Chemical composition and biological activity of Rubus idaeus shoots – a traditional herbal remedy of Eastern Europe, BMC Complement, Alternative Med. 14 (2014) 480. https://doi.org/10.1186/1472-6882-14-480.
[63] K. Wicht, M. Baert, M. Muller, E. Bandini, S. Schipperges, N. von Doehren, G. Desmet, A. de Villiers, F. Lynen, Comprehensive two-dimensional temperature-responsive × reversed phase liquid chromatography for the analysis of wine phenolics, Talanta 236 (2022), 122889. https://doi.org/10.1016/j.talanta.2021.122889.
[64] M. Navarro-Reig, J. Jaumot, A. Baglai, G. Vivó-Truyols, P.J. Schoenmakers, R. Tauler, Untargeted comprehensive two-dimensional liquid chromatography coupled with high-resolution mass spectrometry analysis of rice metabolome using multivariate curve resolution, Anal. Chem. 89 (2017) 7675–7683. https://doi.org/10.1021/acs.analchem.7b01648.
[65] W. Lv, X. Shi, S. Wang, G. Xu, Multidimensional liquid chromatography-mass spectrometry for metabolomic and lipidomic analyses, TrAC, Trends Anal. Chem. 120 (2019), 115302. https://doi.org/10.1016/j.trac.2018.11.001.
[66] G. Theodoridis, H.G. Gika, I.D. Wilson, LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics, TrAC, Trends Anal. Chem. 27 (2008) 251–260. https://doi.org/10.1016/j.trac.2008.01.008.
[67] A.M. King, L.G. Mullin, I.D. Wilson, M. Coen, P.D. Rainville, R.S. Plumb, L.A. Gethings, G. Maker, R. Trengove, Development of a rapid profiling method for the analysis of polar analytes in urine using HILIC–MS and ion mobility enabled HILIC–MS, Metabolomics 15 (2019) 17. https://doi.org/10.1007/s11306-019-1474-9.
[68] B. van de Velde, D. Guillarme, I. Kohler, Supercritical fluid chromatography – mass spectrometry in metabolomics: past, present, and future perspectives, J. Chromatogr. B 1161 (2020), 122444. https://doi.org/10.1016/j.jchromb.2020.122444.
[69] A. Sen, C. Knappy, M.R. Lewis, R.S. Plumb, I.D. Wilson, J.K. Nicholson, N.W. Smith, Analysis of polar urinary metabolites for metabolic phenotyping using supercritical fluid chromatography and mass spectrometry, J. Chromatogr., A 1449 (2016) 141–155. https://doi.org/10.1016/j.chroma.2016.04.040.
[70] L. Si-Hung, T. Bamba, Current state and future perspectives of supercritical fluid chromatography, TrAC, Trends Anal. Chem. 149 (2022), 116550. https://doi.org/10.1016/j.trac.2022.116550.
[71] H.G. Gika, I.D. Wilson, G.A. Theodoridis, LC–MS-based holistic metabolic profiling. Problems, limitations, advantages, and future perspectives, J. Chromatogr. B 966 (2014) 1–6. https://doi.org/10.1016/j.jchromb.2014.01.054.
[72] D.G. Delafield, G. Lu, C.J. Kaminsky, L. Li, High-end ion mobility mass spectrometry: a current review of analytical capacity in omics applications and structural investigations, TrAC, Trends Anal. Chem. 157 (2022), 116761. https://doi.org/10.1016/j.trac.2022.116761.
[73] D. Diamantidou, I. Sampsonidis, T. Liapikos, H. Gika, G. Theodoridis, Liquid Chromatography-Mass Spectrometry metabolite library for metabolomics: evaluating column suitability using a scoring approach, J. Chromatogr. A (2023), 463779.
[74] E. Tsakelidou, C. Virgiliou, L. Valianou, H.G. Gika, N. Raikos, G. Theodoridis, Sample preparation strategies for the effective quantitation of hydrophilic metabolites in serum by multi-targeted HILIC-MS/MS, Metabolites 7 (2017) 13.
[75] C. Virgiliou, N. Fragakis, M. Sotiriadou, V. Vassilikos, S. Gerou, G. Theodoridis, H. Gika, HILIC-MS/MS analysis of adenosine in patient blood, Separations 8 (2021) 222.