Recent applications of high resolution mass spectrometry for the characterization of plant natural products

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Abstract

Recently, the high analytical power provided by the new HRMS instruments (working in the MS or MS/MS mode, stand alone or hyphenated with separation techniques) is making more feasible the characterization of plant secondary metabolites. In this regard, HRMS-based techniques are becoming the technique of choice, since they are capable of answering many questions regarding analytical characterization of secondary metabolites, usually found in very complex matrices, including their structure, composition and concentration in a very fast and sensitive way. In this manuscript we will provide a critical and updated revision of this topic covering the works published in the last 4 years (2015-2018), including the different configurations in which HRMS may be used (stand alone or hyphenated), the data treatment, the strategies for the identification of unknown metabolites, the current software for structure elucidation, as well as the last trends and future outlooks in this hot area of research.

1. Introduction

Plants provide a unique diversity of secondary metabolites that can be exploited for the discovery of new drugs, cosmetic ingredients, nutraceuticals or bio-sourced materials. Natural products are the best sources of chemical diversity for finding new drugs and leads [1]. It is estimated that over 200,000 primary and secondary metabolites may be present in the plant kingdom; for instance, depending on plant genome's complexity a wide diversity of compounds can be found, ranging from around 5000 natural products in a simple model plant such as *Arabidopsis thaliana*, to more than ten thousand compounds in plants with more complex genomes. However, these numbers may be underestimated since many metabolites have not been characterized yet and new publications appear continuously with numerous new structures. In last two decades there was a quick development of mass spectrometric techniques allowing analysis of natural products with differentiated physicochemical properties. Mass spectrometry is currently one of the most versatile and sensitive instrumental methods applied to structural characterization of plant metabolite mixtures [2]. Resolution of the applied mass spectrometers plays an important role in structural studies of mixtures of the target compounds isolated from biological materials. High-resolution analyzers allow obtaining information about elemental composition of the analyzed compounds. Versatility of mass spectrometric methods is based on the possibility of application of different physical phenomena for ionization of analyzed molecules, generation of their fragments and separation of the created ions. In this regard, the application of various mass spectrometric techniques, including different systems of ionization, analysis of both positive and negative ions of analytes, fragmentation of the protonated/deprotonated molecules and in some cases addition of metal ions to the studied compounds before ionization and fragmentation, may improve structural characterization of natural products. High resolution mass spectrometry (HRMS) is not only characterized by its high sensitivity, excellent resolution, and wide detection dynamic range, but also by the

possibility of coupling with chromatographic techniques for online structural identification of each individual peak. Easiness of hyphenation of mass spectrometers with different chromatographic instruments makes it straightforward to perform analyses of compounds present in complex mixtures obtained after extraction of biological material. In many applications combining gas chromatography, liquid chromatography and capillary electrophoresis with mass spectrometry (GC-MS, LC-MS or CE-MS, respectively) different ionization methods can be applied. Furthermore, multidimensional and comprehensive chromatographic techniques (including LCxLC and GCxGC) have also been used to acquire higher peak capacity and resolution. Furthermore, liquid chromatography mass spectrometry (LC-MS) can be interfaced with nuclear magnetic resonance (LC-NMR-MS) for more confirmative structure analysis [3]. Thus, HRMS and its hyphenated techniques have become the major platform for chemical profiling, metabolomics and quality control of plant natural products. The high analytical power provided by the new HRMS instruments (working in the MS or MS/MS mode, stand alone or hyphenated with separation techniques) is making more feasible the characterization of natural products. High resolution mass spectrometry is capable of answering many questions regarding analytical characterization of these compounds, usually found in very complex matrices, including their structure, composition and concentration in a very fast and sensitive way. As a result, HRMS-based techniques are becoming the technique of choice for characterizing these compounds [4-7].

Although the analysis of natural products from plants has been investigated for many years, there is not in the literature a review focusing on the great possibilities and applications of high resolution mass spectrometry, including the methods used (MS, MS/MS, stand alone or hyphenated), and the main natural products and plants investigated in these studies. In this review, we will provide information about this topic and its advances and applications in the last four years (2015-2018) that could be interesting for both the Analytical Chemistry and the

Natural Products communities. Namely, in this article, a revision of the most recent advances and applications of HRMS and its hyphenated techniques for the analysis of plant natural products, including but not limited to one-dimensional (1D) chromatography, multidimensional chromatography (MDC) coupled to MS, ambient ionization MS, mass spectral databases and data treatment will be presented. Only papers focused on plant natural products are considered, discarding other natural sources (e.g., microorganisms, fungi or marine origin) in order to fulfil the constraint about the number of references marked by the journal. As a summary, Table 1 shows the selected stand-alone and hyphenated HRMS applications published in the period 2015-2018 that will be further discussed in the present work.

2. Analysis of natural secondary metabolites using hyphenated HRMS.

The hyphenation of HRMS with high throughput separation techniques is a powerful strategy for the sensitive screening analysis and rapid identification of secondary metabolites in complex natural matrices. Chromatographic techniques coupled to HRMS, mostly via liquid chromatography (LC-HRMS) and, in minor extent, gas chromatography (GC-HRMS), are the most popular options for characterization purposes in plant natural products research. These hyphenated approaches significantly increase the speed of analysis, improving selectivity, resolution and efficiency, and provide improved structural determination capabilities compared to methods based on IR and Raman spectroscopy [5]. On the other hand, GC-HRMS allows the detection of volatile or derivatized non-volatile metabolites at lower concentrations compared to other techniques for structural characterization, such as NMR [8]. Typical HRMS analyzers coupled to chromatographic separation techniques involve mainly hybrid quadrupole time-of-flight (Q-TOF) MS instruments; although other hybrid configurations such as TOF-TOF or ion trap-TOF (IT-TOF) are also reported. Furthermore, high-resolution ion trap instruments such as the hybrid quadrupole-Orbitrap (Q-Orbitrap), and hybrid linear ion trap-Orbitrap (LTQ-

Orbitrap) mass analyzers are more and more frequently used for secondary metabolites analysis [9].

Application of the abovementioned HRMS instruments and their hyphenated techniques for the analysis of natural secondary metabolites, as well as the most widespread ionization sources used for each hyphenated HRMS configuration, will be reviewed and compared in the following subsections.

2.1 Ionization techniques

The popularity of HRMS in natural products research can be mainly attributed to its versatility to adopt multiple configurations with interchangeable ionization sources and sophisticated data acquisition capabilities, which allows the determination and structural elucidation of a broad variety of metabolites with diverse physicochemical properties (e.g. polarity, volatility, thermolability, molecular weight). In this regard, the ionization source plays a crucial role in the targeted and untargeted analysis of natural products, since the detectability of the secondary metabolites of interest is strongly influenced by the employed ionization mode; the interface in hyphenated HRMS techniques.

Electrospray (ESI) or heated electrospray (HESI) ionization source is the most popular and widespread used interface in HRMS instruments coupled to LC techniques. The heated probe is reported to provide better desolvation and enhanced sensitivity, due to the cleaner nozzle profile produced by the heating effect, which improves electrostatic fields [10]. Depending on the pH of the target compound and the ability of the target structure to be charged in positive or negative mode, ESI(+) or ESI(-) ionization must be selected accordingly for improved sensitivity. For multi-analyte methods, switching between positive and negative modes is frequently recommended to cover a wider range of chemical structures. ESI-based ambient ionization techniques are intended for the analysis of both small molecules and

macromolecules exhibiting moderate polarity, due to the formation of single and multiple charged molecular ions [7], whereas ambient ionization sources based on atmospheric pressure chemical ionization (APCI) are mainly used for the analysis of less polar and small molecules, with the generation of single charged molecular ions. Thus, the selection of the most appropriate ionization method according to the polarity and molecular weight of target metabolites plays an important role on their detectability. A broad variety of secondary metabolites in plant natural products, including phenolics, flavonoids, terpenoids, alkaloids, coumarin, and saponins, among others, have been analyzed using ESI-related ambient ionization techniques coupled to different HRMS instruments such as TOF [11-13], Q-TOF [14-21], IT-TOF [22-24], LTQ-Orbitrap [25-27], Q-Orbitrap or Fourier-transform ion cyclotron resonance (FT-ICR) [28].

Alternatively, electronic impact (EI) ionization has historically been the standard ionization interface in MS instruments hyphenated to GC. Due to the highly reproducible mass fragmentation obtained by EI, the hyphenation GC-EI-HRMS represents a powerful tool and the first choice for identification and structure elucidation of unknown volatile and semivolatile organic compounds [4]. The standardized fragmentation, fixing the ionization energy at 70 eV allows spectral patterns that can be easily and systematically searched in well-established MS databases (e.g. NIST MS database, Fiehn Lib, Wiley, etc.), making this technique very attractive for unknown's analysis in natural extracts [7]. Nowadays, GC-EI-(Q)TOF is one of the most frequent HRMS hyphenations [14, 29-31], as will be discussed below.

Ambient ionization techniques based on desorption phenomena, such as matrix-assisted laser desorption/ionization (MALDI) or direct analysis in real time (DART) have been mainly reported for stand-alone configurations of HRMS instruments and will be reviewed below in section 3.

2.2 TOF-MS methods

HRMS instruments based on TOF-MS analyzers are the most extensively reported in literature for the analysis of plant natural products. In particular, the hybrid Q-TOF-MS instruments have been largely adopted as powerful tools for untargeted analysis of complex plant extracts, due to their capability of providing accurate mass data, and structural information from HR-MS/MS fragmentation [5, 7]. High pressure liquid chromatography (HPLC) has been traditionally used as conventional separation technique coupled to HRMS for secondary metabolites analysis, although ultra-HPLC (UPLC) is nowadays implemented in many analytical laboratories, providing higher throughput analysis and improved chromatographic resolution. For metabolite profiling studies, HPLC or UPLC coupled to Q-TOF has become increasingly popular for the characterization of complex biological samples, combining high separation efficiency with high structural elucidation capacity, generating thousands of MS features per sample. For instance, a comprehensive characterization of goldenberry calyx extracts was reported by Ballesteros-Vivas et al. [14] applying a UPLC-ESI-Q-TOF-MS/MS method, operating both in ESI+ and ESI- mode. More than fifty phytochemicals were tentatively identified, including major phenolic components, withanolides (C₂₈-isoprenoids), and a large family of anti-inflammatory sucrose esters, applying an integrated identification strategy (See strategies for unknown identification below in section 4.2). A targeted approach was proposed by Peng et al. [32] for the metabolite profiling of steroidal glycosides in the rhizome of *Aspidistra sichuanensis* by UPLC-Q-TOF-MS^E (MSE, simultaneous acquisition of MS spectra at low and high collision energies). Based on the fragmentation pathways of six reference steroidal saponins, the structures of 60 metabolites were tentatively identified.

Several HRMS-based methods for the rapid identification of certain groups of secondary metabolites in plant natural extracts have been reported applying hybrid Q-TOF-MS systems coupled to (U)HPLC separation techniques. Thus, new amorfrutin derivatives from *Amorpha*

fruticose were determined through a screening strategy based on precursor ion scan monitoring mode [17], whereas a rapid discovery strategy of cyclopamine alkaloids from *Fritillaria* and *Veratrum* plants was proposed based on characteristic fragment ions of previously characterized reference compounds [33]. Other untargeted methods to screen for agonists of enzymatic receptors (e.g. peroxisome proliferator-activated receptor- γ) from natural products propose the hyphenation of LC-Q-TOF-MS instruments with immobilized fusion protein affinity chromatography to achieve rapid multicomponent screening [21].

In LC-Q-TOF-MS for the profiling analysis of phenolic antioxidants, hyphenation to DAD detectors has been commonly employed for further peak annotation or dereplication, providing valuable on-line structural information based on retention times, UV spectra, exact mass, and fragmentation ions from MS/MS spectra. On-line HPLC-DAD-Q-TOF-MS/MS system was applied for the rapid chemical profiling of flavonoids from *Fructus aurantii immaturus* [16], minor antioxidants from leaves of *Malus doumeri* [34] and unknown and minor isomeric chlorogenic acids in the buds of *Lonicera macranthoides* [35], whereas alternative off-line configurations (HPLC–UV/Q-TOF-MS) were proposed for the rapid screening of brain-penetrable antioxidants from natural products [18] and for active fragments-guided discovery of selective tropane alkaloids drugs [19]. Other screening methods for the determination of nitric oxide scavengers [36], and other active and functional compound combinations [37] evidenced the broad applicability of the hyphenated DAD-Q-TOF systems. Figure 1 illustrates the complementary UPLC-DAD, and UPLC-Q-TOF-MS information for tropane alkaloids analysis from *Physochlaina infundibularis* (HSS in China).

Besides metabolite profiling, several (U)HPLC-hyphenated Q-TOF-MS techniques have been reported for the rapid and robust screening of new bioactive compounds from complex natural product matrices. The “so-called” dereplication methods aims for testing sample mixtures that show bioactivity in screening bio-assays, in order to differentiate the novel compounds from

active substances that have already been studied [4]. These methods are particularly relevant for drug discovery and pharmaceutical research and can be used applying a targeted or an untargeted approach. In this regards, applying different dereplication strategies (e.g. HPLC/bioassay-guided fractionation, molecular networking and docking), Abbas-Mohammad et al. [15] identified flavonoids and furanocoumarine-type compounds with acetylcholinesterase-inhibitory activity in Iranian plants. A HPLC-QTOF-MS-guided isolation approach was proposed by Jiang et al for the rapid discovery and targeted isolation of novel hepatoprotective triterpenoid saponins from the methanol extract of *Celosiae Semen* [38]. Other bioactivity-based screening methods using ESI-QTOF-MS hyphenated instruments were recently reported for the target cell-based neuroactivity screening of *Panax ginseng* [39] and for the identification of multiple constituents in a Chinese medicine formula from plants (Xiao Chai Hu Decoction) by bioactivity-guided fractionation [40].

Hybrid linear ion trap (LIT) coupled to TOF-MS analyzer combines the MSⁿ capabilities of ion trap and the excellent resolution of a time-of-flight, highly appreciated properties in structural elucidation studies [22, 24, 41]. Using MS/MS fragmentation by collision induced dissociation (CID), Nakata et al. [22] studied the fragmentation behavior of 2 flavones and 4 isoflavones by HPLC-IT-TOF-MS/MS. Isotopically labeled isoflavones allowed the mechanistic understanding of characteristic isoflavone fragmentation patterns. A similar approach was applied by Wang et al. [24], using notoginsenosides as model compounds to optimize collision energy on the basis of accurate MS² and MS³ measurements, demonstrating that the qualitative capability of HPLC-IT-TOF-MS/MS instruments was greatly influenced by CID energies.

Two-dimensional (2D) LC separation methods coupled to HRMS show great potential for the analysis of complex natural extracts, especially for exhaustive metabolome profiling analysis where high resolution for a greater number of compounds is needed. In this regards, the use of

an off-line hydrophilic interaction x reversed-phase (HILIC × RP) 2D-HPLC-DAD-Q-TOF-MS method has shown to greatly improve the separation and characterization of natural products in Ginkgo biloba extract, detecting 125 compounds with a 69.5% estimated orthogonality in the 2D-LC system [42].

The hyphenation of Q-TOF systems with gas chromatography (GC) can also be considered a powerful tool and the first choice for identification and structure elucidation of unknown volatile and semivolatile organic compounds [43, 44]. Although standard spectral libraries (NIST and Wiley) are available for GC–MS, the compounds identification is still difficult using low-resolution MS. GC-Q-TOF(MS) is becoming more popular in natural products research due to its high sensitivity in full-scan MS mode together with high resolving power and accurate-mass measurements, which provides high confidence of identification [7]. GC-Q-TOF(MS) has been recently applied to the metabolite profiling of *Mimosa pudica* L. [29], dereplication studies with spectral deconvolution ratio analysis [30], and for non-polar natural products analysis [31], among others. In these works, several derivatization procedures were used to efficiently separate relatively polar metabolites by GC-MS. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used as silylating agent by Tasnuva et al. [29] for the metabolite profiling of *Mimosa pudica* L, whereas a two-steps derivatization procedure was applied by Carnevale et al. [30], involving methoximation to protect aldehydes and ketones, and to inhibit the ring formation of reducing sugars, followed by silylation reactions with N-methyl-N-trimethylsilyltrifluoroacetamine (MSTFA) to protect different acidic protons. However, the derivatization may lead to increased complexity due to incomplete reactions and the formation of multiple derivatization by-products [4]. Therefore, derivatization is sometimes avoided in GC-Q-TOF(MS) analysis [14, 31].

Both GC and LC-hyphenated Q-TOF platforms are highly complementary alternative tools, providing MS and MS/MS data for metabolites with different physicochemical properties, and

generally, only a limited number of compounds are simultaneously detected by both platforms, increasing the coverage in metabolite profiling and metabolome studies [4]. In this regards, the great potential of integrating identification strategies based on HRMS data obtained from complementary LC- and GC-Q-TOF-MS(/MS) platforms has been recently reported as a powerful identification tool for improving our understanding on the phytochemical composition of natural extracts [14].

2.3 Orbitrap MS methods

Orbitrap-MS detectors are HRMS analyzers belonging to the Fourier transform mass spectrometry (FT-MS) family, characterized by increased acquisition speed, higher resolving power, mass accuracy, and sensitivity [45]. Since the introduction of the first commercial hybrid LTQ-Orbitrap-MS in 2005, several extensions of this technology were commercialized, being widely distributed nowadays in analytical laboratories. For the LTQ-Orbitrap, the high resolution mass spectrum is acquired with an Orbitrap, and tandem mass spectrometry (MS/MS) can be obtained with ion trap (collision induced dissociation, CID) or Orbitrap (higher-energy collisional dissociation, HCD). For the Q-Exactive, the Orbitrap is used for acquiring both MS and MS/MS spectra [46]. Although Orbitrap instruments have originally found great applications in proteomics analysis, where very high resolution is required, several hyphenated LTQ-Orbitrap-MS methods, mainly coupled to (U)HPLC-based techniques, have also been reported for small compounds identification [25, 47-49] and metabolite profiling [26, 50, 51], as well as for metabolome [52] or dereplication studies of natural products in complex plant extracts [27].

In the last decade, Q-Orbitrap-MS instruments became popular with the introduction of Q-Exactive family, which exhibit high performance, not only for proteomic purposes but also for metabolomics and high throughput screening analysis of secondary metabolites. Hybrid

Q-Orbitrap-MS instruments combine high mass resolving power, provided by orbitrap mass analyzer, with the selectivity of the quadrupole. Thus, multiple precursor ions can be fragmented in a high-energy collision cell, and the product ions can be accurately detected with very low mass error (<3 ppm), in a wide dynamic range of concentrations [53]. Q-Orbitrap-MS analyzer have been recently reported in several applications related to natural products characterization, mainly hyphenated to liquid chromatography. Nardin et al. [54] demonstrated the versatility of Q-Orbitrap systems to perform accurate quantitation of alkaloids and untargeted screening of further alkaloids in herbal extracts. On-line coupling of UV detectors with Q-Orbitrap were also reported in characterization [55], metabolic [56] and dereplication [57] studies, demonstrating that (U)HPLC-DAD-Q-Orbitrap-MS hyphenation can effectively detect and quantify small organic compounds such as phenolics and sesquiterpene lactones. Móricz et al. [58] reported the benefit of high-performance thin-layer chromatography (HPTLC)-hyphenations in the discovery and characterization of active natural compounds. Non-targeted screening of sunflower leaf extract by HPTLC-ESI-Q-Orbitrap-MS allowed the targeted characterization of components exhibiting antioxidant activity, and diterpenes with antibacterial and/or cholinesterase enzyme inhibitory effects. Other reported applications using Q-Orbitrap systems involve the isolation and characterization of cytotoxic triterpenoids against a breast cancer cell line [59], untargeted profiling of glucosinolates in cauliflower [60], metabolomics of herbal medicinal products and human gut bacteria interactions [61], characterization of antioxidants [62] and potential enzyme inhibitory compounds linked to Alzheimer's disease (e.g. mono-C-glycosyl flavones) [63].

3. Analysis of natural secondary metabolites using stand-alone HRMS instruments

3.1 MALDI-HRMS methods

Matrix-assisted laser desorption/ionization is a soft ionization technique used in the analysis of high molecular weight compound. When combined with TOF mass analyzer, mass spectra of proteins larger than 100 000 Da can be obtained; consequently MALDI-TOF represents one of the major analytical tools in the modern life sciences and in polymer science [64]. According to Silva et al. [65], the ionization process in MALDI can be explained through three basic steps: 1) the incorporation and isolation of analytes in a matrix, 2) the excitation of the matrix producing the plume by physics desorption/ablation, and 3) the ionization of the analytes by ion-molecules reactions. Recent reviews [65, 66] discuss MALDI theory, instrumentation, matrices and applications related to natural product analysis such as carbohydrates and glycoconjugates.

MALDI-TOF is an important tool for the structural elucidation of compounds with high-molecular mass and/or high polymerization degree. De Brum Vieira et al. isolated and characterized bidesmosic saponins with high anti-trichomonas activity from *Manilkara rufula* using MALDI-LIFT-TOF, among other spectroscopic techniques [67]. MALDI-LIFT-TOF showed fragment ions from saccharide ester chains and triterpenic aglycone (protobassic acid) from saponins. Subsequently, MS/MS analysis confirmed sugar losses and the number and positions of these units, allowing the identification of twelve bidesmosic saponins in *M. rufula* extract. Using the same approach, Demarque et al. characterized gastroprotective condensed tannins from *Stryphnodendron rotundifolium* Mart. [68]. Due to the high polymerization degree of condensed tannins, its structural elucidation is difficult; MALDI allows identifying the fragmentation pattern followed by Retro-Diels-Alder (RDA; -136 Da) and Remote-hydrogen rearrangement (RHR; -152 Da) units (Figure 2). Following this pattern is possible to identify the monomers constituting the different oligomers, thus providing reliable identification of units and degree of polymerization.

MALDI-TOF has also been used to characterize different batches during natural products production and to ensure their quality control and safety. In this sense, Cibulski et al. compared a semi purified aqueous extract (AE) and saponin-enriched fraction (QB-80) from *Quillaja brasiliensis* leaves with Quil A®, a commercial fraction from *Quillaja saponaria* bark, regarding performance as vaccine adjuvants and saponins profiles [69]. The spectra patterns of the *Q. brasiliensis* and *Q. saponaria* saponins were obtained by MALDI-TOF analysis, showing that both AE and QB-80 presented noteworthy chemical similarities to Quil A®. Consequently, *Q. brasiliensis* fractions are a possible alternative to commercial Quil A®. This work demonstrates that MALDI-TOF analysis is a rapid tool to obtain standards and to achieve reproducible protocols for extraction, purification and characterization of natural products. On the other hand, Cornelio et al. [70] developed a quick and efficient method based on MALDI-LTQ to confirm the presence of meliatoxins A₁/B₁ and A₂/B₂, two toxic limonoids, in the *Melia azedarach* extract, a promising natural product for sustainable pest management systems. The MS and MS/MS fragmentation patterns serve as fingerprints of meliatoxins and are useful for their identification in other natural products.

MALDI-TOF has also been used to establish tissue distribution of metabolites. In this sense, Hwang et al. investigated the biodistribution of gomisin H, a natural product derived from *Schisandra chinensis*, in mouse organs using MALDI-TOF after intravenous injection of the compound [71]. Each mouse isolated organ (brain, heart, lung, kidney, spleen, liver, and pancreas) was extracted with ethyl acetate and the extract was analyzed by MALDI-TOF in the presence of gomisin J (used as internal standard). The mass spectrum was used to derive quantitative information on gomisin H by comparing the peak intensities of the gomisin H and gomisin J. The organ extracts were incorporated in a gold nanoparticle (AuNPs) matrix for the desorption/ablation and ionization processes. Results showed that gomosin H was accumulated

in the mouse liver. Thereby, this work demonstrated multiplexing capability of MALDI-TOF to analyze biodistribution of natural products and pharmacokinetic interactions in an *in vivo* model. Moreover, MALDI-TOF is a powerful tool to confirm the identity of isolated compounds from natural products. In this sense, Altemimi et al. isolated and quantified some flavonoids (quercetin, myricetin, and rutin) from peaches and pumpkins fruits by TLC and a densitometric quantification method [72]. The TLC spots were analysed by MALDI-TOF and mass spectra allowed confirming the chemical identity of isolated compounds.

3.2 ESI-HRMS methods

ESI-MS is one of the most popular stand-alone techniques for chemical characterization of extracts and compounds isolated from natural sources. Kumar et al. developed a rapid identification method of limonoids from *Cipadessa baccifera* and *Xylocarpus granatum* crude extracts using ESI(+)-q-TOF-MS/MS [73]. The identification was based on CID-MS/MS fragmentation pattern of common limonoids (methyl angolensate type- and mexicanolide type- limonoids) previously studied. This generalization of the structure- fragmentation relationship was applied for the identification of compounds in crude extracts of these plants and resulted in the putative identification of 27 limonoids (Figure 3). On the other hand, Phansalkar et al. obtained oligomeric proanthocyanidins fractions from grape seeds extracts using centrifugal partition chromatography (CPC) [74]. CPC fractions were analyzed by ESI-IT-TOF and the mass spectrum information enabled the classification of the fractions according to their polymerization and galloylation degrees. This classification allows subsequently orthogonal chromatographic separation steps for the isolation, purification and characterization of some oligomeric proanthocyanidins. Cui et al. [75], recently isolated and characterized steroidal saponins from *Aspidistra typica* rhizomes using spectroscopic techniques and HRMS. MS information was obtained by ESI-Q-TOF and the spectra showed multiple fragments

attributable to the saponin aglycone, sequential losses of monosaccharide residues and molecules of water. With this information and combining with NMR experiments (COSY, HSQC and HMBC), it was possible to establish the chemical structure of the new eleven furostanol saponins and one spirostanol saponin.

Due to the unparalleled resolution and sub-ppm accuracy in mass determination and its simultaneous capacity for detecting multiple compounds without previous separation, recently, Park et al. [28] incorporated the ultra-high-resolution FT-ICR-MS to a method for detecting antioxidant compounds from *Morus alba* fruit extract. ESI-FT-ICR-MS was used to determine the molecular formulae of compounds in the extract and to correlate the mass profiles with antioxidant activity according to SCAMP method (Scaling of correlations between activity and mass profiles).

ESI-HRMS not only contributes to accurate mass determination and to structure elucidation, but also to evaluate regiochemistry and stereochemistry of organic compounds. In this way, Hernandez et al. [76] studied the energy threshold for the monocaffeoyl and dicaffeoyl quinic acids (from coffee extracts) fragmentation by direct infusion resolved mass spectrometry (ER-MS) using ESI-Q-TOF and ESI-IT. In ER-MS, the break down graphs (BDGs) of the organic compounds are plotted, showing the decrease of the precursor ion intensity against the energy collision. Integration of BDGs over the internal energy distribution of ions at several energy collision resulted in the so-called fragmentation efficiency curves[76]. Numerical parameter of efficiency curves allows determination of regiochemistry of fragment ions. In this study Q-TOF data showed a better separation of fragmentation energy.

Most popular stand-alone ESI-HRMS applications are related to mass accuracy determination of isolated compounds for chemical structure elucidation. Combination with spectroscopic methods, including one and two-dimensional NMR, UV-Vis, FT-IR, X-ray crystallography, circular dichroism, among others, is a methodology widely employed for this purpose.

Following this approach several authors have identified new compounds from vegetal sources in recent years. With the help of ESI-TOF or ESI-Q-TOF, as complementary technique, several classes of new compounds have been characterized including nortriterpenoids [77], unsaturated cyclic ketones [78], daphnane diterpenoid orthoesters and polycephalones [79], sesquiterpenes [80] and bisbibenzyls [81]. Using Orbitrap and LTQ-Orbitrap the exact mass of new compounds, such as triterpenoids [59], cleordane diterpenoids [82], β -Dihydroagarofuran derivatives [83] and isoflavonoids [84] have been also determined. Ultra-high-resolution FT-ICR-MS and FT-MS have been also applied for identification of phorbol ester [85] and glycyuralin derivatives [86].

3.3 DART-HRMS methods

Direct analysis in real time (DART) is an ambient plasma ionization method based on the reactions of long-lived electronic excited-state atoms or vibronic excited-state molecules with the analytes and atmospheric gases [87]. The ion formation principle in a DART source is based on the ambient air–chemical reactivity activated by a gas stream of species generated in an excited metastable state [88]. In recent years, DART-MS has been implemented as analytical tool in several research fields such as food chemistry, environmental contaminants analysis, clinical and pharmaceutical studies and phytochemistry. DART-MS allows the analysis of solids, liquids and gases with minimal or no sample preparation. Although in the phytochemistry field, DART is usually coupled to low resolution instruments, new DART-HRMS applications have been developed for the analysis and identification of secondary metabolites from plant sources [89]. Shen et al. reviewed applications of DART in the field of herbal medicine analysis, including compound detection, species identification, metabolites profiling and initial quantification [90]. The most common HRMS instrument type coupled to DART is Q-TOF, although Orbitrap has also been hyphenated. Several families of secondary metabolites have

been studied by DART-HRMS, including phenolic compounds, alkaloids, saccharides and iridoid glycosides. Recent works show the great potential and efficacy of DART-HRMS for rapid screening and bio-distribution studies of compounds in different plant tissues [91, 92], fingerprint studies of plant species [93-95], as well as for quality control evaluation of herbal products, such as herbal teas [96] and cannabis derivatives [97].

4. Data treatment

4.1 Mass spectral databases

Although several reviews can be found in the literature concerning mass spectra databases and libraries [44, 98-102], in the present work we will focus on available databases containing HRMS information with special emphasis on plant metabolites:

- ChemSpider (<http://www.chemspider.com>) is a free chemical structure database providing fast text and structure search access to over 67 million structures from hundreds of data sources [103]. ChemSpider provides information about physicochemical, bioactivity and mass spectra properties of known metabolites, useful for chemical identification processes.
- METLIN is a high-resolution tandem mass spectrometry database created in 2003 [104]. METLIN contains information about 1 million compounds including lipids, amino acids, carbohydrates, toxins and natural products. METLIN's MS and MS/MS data has been obtained in a ESI(+/-)-Q-TOF system and the information covers isotopic m/z shifts. Recently, METLIN has incorporated *in silico* MS/MS spectra based on learning algorithms. Human Metabolome Database (HMDB) is a free database of human metabolites although also includes secondary metabolites from natural products associated to human metabolism. HMDB contains chemical, clinical and molecular biology data of more than 100,000 metabolites, including food components and food

additives [105]. For chemical characterization purposes, HMDB allows to query GC-MS and LC-MS spectrometry data.

- MassBank is a comprehensive database with data acquired in HRMS through GC-MS and LC-MS systems [102]. MassBank includes information about name, structure, exact mass, peaks, neutral losses of more than 11,000 compounds. In addition, MassBank has a search tool by ionization source (i.e. ESI, APCI, MALDI), and mass analyzer (i.e. Q-TOF, ITFT, TOF).
- NIST database is a commercial database developed by National Institute of Standards and Technology and supplied by John Wiley and Sons [100]. NIST was originally developed for EI-MS, however, it now includes data derived from several soft ionization and mass analysis systems [44]. NIST contains more than 190,000 MS/MS spectra of small compounds.
- Wiley Registry database includes more than 700,000 MS/MS spectra and 683,000 chemical structures: pharmaceutical compounds, drugs, pesticides and natural products [98]. The MS/MS spectra were obtained with ESI(+/-)-HRMS instruments at different collision energies in the range of 5-50 eV [44]. Wiley Registry is one of the most comprehensive mass spectral libraries focused on the identification of unknowns compounds in untargeted analysis.
- mzCloud is a free database with more than 8,000 and almost 3,000,000 spectra [106]. mzCloud contains mass spectral information on authentic chemical standards measured on Orbitrap instruments [100]. These include raw and calibrated MS spectra and MS/MS spectra displayed at various collision energies. Spectral peaks are structurally annotated using one or more in silico prediction methods.

4.2 Strategies for the identification of unknown metabolites

Searching in MS databases is nowadays one of the more straightforward approaches to confidently identify secondary metabolites. However, although MS/MS fragmentation databases have been continuously growing during the last decade, both in coverage and diversity, annotation of unknown phytochemicals is still a challenging task for metabolite profiling, dereplication or plant metabolome studies [44]. Unlike the high reproducibility of spectral patterns in EI-MS databases via GC-EI-MS application working at a standard ionization energy at (70 eV), fragmentation spectra obtained by different LC-MS/MS platforms are instrument-specific and may vary depending on MS/MS instrument type and configuration (Q-TOF, IT-TOF, LTQ-Orbitrap, Q-Orbitrap, FTICR), ionization method (ESI+/-, MALDI), or collision energy (collision-induced dissociation, CID; or higher energy collisional dissociation, HCD), among other factors. Therefore, complementary strategies for the identification of unknown or unreported metabolites need to be applied, based on the analytical capabilities of the employed HRMS instrument [4, 43, 44].

HRMS acquisition strategies

HRMS data obtained in full scan MS mode provide exact m/z values that can be used to generate chemical formulas with high mass accuracy and to calculate the number of double bond equivalents (or degree of unsaturation; DBE) [9]. An acquisition strategy based on precursor ion-scan mode screening followed by formula calculation was shown to provide rapid identification of unknown amorfrutins in *A. fruticosa*. and can be useful in the discover of other trace natural products and metabolites [17]. Full-scan information can be obtained with all HRMS platforms at a certain resolution power and mass resolution according to the specifications of the instrument; however, to improve annotation, additional structural information based on HR-MS/MS spectra is required. Information regarding the chemical

structure from MS/MS fragmentation can be obtained varying some instrumental parameters and operating in different acquisition modes or with different hybrid HRMS configurations. The development of high throughput mass analyzers with increased acquisition speed and the emergence of hybrid HRMS instruments, gave rise to automated MS/MS data acquisition modes that has greatly increased the MS information obtained in a single analytical run [4]; namely, data-dependent (DDA) and data-independent (DDI) acquisition modes. In DDA, a precursor ion peak with high abundance is selected in an initial MS scan event, and fragmented in a subsequent MS/MS scan event by CID or HCD); the latter in orbitrap instruments. DDA operates by triggering the fragmentation step on the basis of predefined criteria, such as the intensity threshold and number of selected precursor ions or dynamic exclusion (limiting reoccurring precursor ions) [44]. Despite the advantages of this acquisition mode related to its high throughput analytical capabilities, the possibility of triggering MS/MS fragmentation of highly abundant ions corresponding to irrelevant compounds from the background or from the sample matrix is a limitation, that can overlook relevant structural information of metabolites of interest. In such case, a re-analysis in targeted DDA mode is required. Unlike DDA, where MS/MS spectra contain information about the selected precursor ion, in DDI all precursor ions detected in MS scan mode within a predefined mass range undergo fragmentation, providing full scan MS/MS spectra. This automated acquisition mode can be operated in several hybrid HRMS instruments under different strategies, such as MS^E (Waters Corporation), All-Ions MS/MS fragmentation (AIF, Agilent Technologies), broadband CID (bbCID; Bruker), multiplexed MS/MS data-independent acquisition (MSX-DIA; Thermo Fisher Scientific) or sequential window acquisition of all theoretical mass spectra (SWATH; Sciex), depending on the manufacturer [9].

Since collision energy is one of the most important factors in acquiring MS/MS information, a fragmentation behavior study under different stages of MSⁿ and variation of CID energy

was reported for notoginsenosides as model compounds in natural products [24]. Under this approach, a methodology for the collision energy optimization was proposed to identify drug metabolites and determine trace multi-components in natural products.

Considering the lack of reproducibility of spectral patterns in MS/MS databases, even with CID and HCD spectra obtained from a variety of LC-MS/MS instruments, automatic identification might be not so straightforward for some unknowns. In such case, fragmentation mechanisms of reference compounds can be useful to build an in-house MS/MS spectral library of analogous compounds, and propose an elucidation strategy for structurally related target metabolites. Thus, an integrated characterization strategy for the systematic discovery of indole alkaloids was developed based on in-house library data obtained by UPLC-IT-Q-Orbitrap [48]. A quick identification scheme was also reported for different camptothecin skeletons, based on the MS/MS data of four kinds of reference camptothecins [107].

Other strategies based on the combined application of soft ionization in both ESI(+) and ESI(-) modes led to different CID MS/MS fragmentation mechanisms for the same metabolite, providing complementary structural information. This strategy has been reviewed for the structural characterization of flavonoids glycoconjugates, widespread secondary metabolites in plant extracts [2]. The different MS/MS information obtained from CID fragmentation of protonated or deprotonated metabolites can help to differentiate between O- and C- glycosides of flavonoids. However, some limitations must be overcome with complementary elucidation techniques and further fragmentation strategies, regarding the accurate determination of the exact nature of the sugars and the substituent position in the aglycone moiety. A similar strategy was recently reported to characterize withanolide (C₂₈ ergostane-type steroids) compounds in a natural extract of *P. peruviana* [14]. Isobaric withanolides with differences in the hydroxylation of the lactone and ergostane moieties can be determined comparing positive and negative CID MS/MS spectra.

575 *Post-acquisition data mining strategies*

576 The huge amount of datasets generated by HRMS measurements pose a great challenge due
577 to the need of processing thousands of MS features to extract useful information. In this
578 regard, several data mining strategies have emerged to facilitate the post-acquisition data
579 processing. Systematic approaches such as mass defect filtering (MDF), diagnostic fragment
580 ion filtering (DPIF), background subtraction filtering (BSF), or neutral loss filtering (NLF),
581 have been reported [108]. MDF is a screening approach based on the difference between a
582 compound's exact mass and its nominal mass, that allow filtering metabolites with the same
583 core substructure, within specific mass ranges. In addition, metabolites exhibiting very
584 similar fragmentation behaviour share the same product ions and neutral loss fragments in the
585 MS/MS spectra. Therefore, information provided by product ions or neutral loss fragments
586 allow the detection of compounds with similar core substructures or functional groups. The
587 implementation of these filtering strategies allows to systematically screen HRMS data
588 looking for families of compounds. These filtering strategies can be easily implemented using
589 open-source software such as MZmine to extract MS features from the raw UPLC–MS data,
590 producing a peak list which can be processed according to the proposed workflow depicted
591 in Figure 4 [108]. Several authors have reported strategies for post-acquisition processing of
592 HRMS data obtained from natural products [14, 108, 109]. The characterization of alkaloids in
593 *Uncaria rhynchophylla* was achieved using mass defect filtering and neutral loss filtering [48].
594 After convenient isolation, direct-infusion fragmentation behaviors were studied to obtain
595 diagnostic structural information for rapid characterization of related alkaloids. Figure 5
596 illustrates the proposed integrated strategy for the discovery of new bioactive molecules from
597 herbal medicines. The strategy using fragmentation behaviors and structural information from
598 diagnostic ions or neutral loss was also used for the discovery of selective tropane alkaloids
599 [19]. These approaches were shown to be useful in active fragments-guided drug discovery

studies, to explore the relationship between receptors and potential inhibitors. Taking advantage of the high-resolution MS/MS spectra from Q-Exactive MS, Quiao et al. [53] proposed a targeted strategy for the chemical profiling of *Scutellaria* compounds, capable of distinguishing isobaric ions based on key ion filtering from the obtained diagnostic MS/MS fragment ions.

The application of the abovementioned strategies may result in the tentative identification of the detected metabolites. For unambiguous identification of tentatively identified compounds with uncertain annotation, confirmation with reference standard is required. Alternatively, isolation, purification and “de novo” identification by NMR or synthesis of the putative structure is necessary for further confirmation [4]. In fact, according to Chemical Analysis Working Group (CAWG) of the Metabolomics Standards Initiative (MSI), the level of confidence in the identification for metabolites reported should be based on the following four-level system: Level 1 (identified compound), Levels 2 and 3 (putatively annotated compounds and compound classes), and Level 4 (unidentified or unclassified metabolites which nevertheless can be differentiated based upon spectral data) [110]. This recommended rules of general consensus on reporting standards applies to the research community working on metabolomics studies. For natural products identified for the first time, accurate mass data from HRMS, along with NMR (¹H and ¹³C) data and stereochemistry confirmation by circular dichroism and/or alpha D values are required [111].

4.3 Software for structure elucidation

To overcome the limitations associated to the inter-instrument variability of HR-MS/MS data, incomplete MS/MS databases, and the lack of authentic standards, several computational annotation tools based on MS training data of authentic standards provide some help on the large-scale identification of metabolites from natural sources. Some public or commercial

websites and software packages are available nowadays, including Mass Frontier (HighChem), MS fragmentor (ACD lab), Fragment Identifier (FiD), MetFrag and Metfusion [7]. These tools work applying fragmentation rules obtained from literature, or from authentic standards. XCMS2 is a free software that can directly search in METLIN online data acquired on an QTOF instrument with collision energies from 0 to 40 eV [44]. Other publically available software include the Wiley MSforID search algorithm (available with the MSforID library); the SMILEMS software, that allows working with multiple MS/MS and MS_n databases; or the Mass++ software that supports MS/MS search of MassBank spectra and data processing [44]. MZmine is an open-source software for MS data processing from different hyphenated strategies although more focused on LC-MS data. MZmine covers the entire LC-MS data analysis workflow with demonstrated great possibilities for MS data filtering and metabolomics. MZmine supports mzML and mzXML data, as well as Thermo and Waters RAW data files. Alternatively, Progenesis QI is a commercially available software for metabolomics profiling data, allowing MS/MS search against reference libraries (e.g. MassBank, METLIN). Progenesis supports multi-vendor MS data formats (Agilent, Bruker, SCIEX, Thermo), as well as mzML and mzXML data [44]. The UNIFI informatics platform (Waters) integrates data acquisition, data mining and database searching. The integration of UPLC-QTOF-MS^E analysis with the UNIFI informatics platform provides a high throughput solution for the routine analysis of functional compounds in plant natural products [112].

Other specific computational tools such as Automated Mass Spectral Deconvolution and Identification System software (AMDIS) and Ratio Analysis of Mass Spectrometry deconvolution (RAMSY) have been developed for deconvolution of complex spectra and matching of a purified spectrum to a reference spectrum to improve identification of unknown metabolites [4]. RAMSY deconvolution algorithm in combination with AMDIS was shown to

improve the spectral identification workflow in dereplication studies of natural products from GC-MS data [30].

5. Conclusions and Future Outlooks

Plant natural products have attracted more and more attention since they can exhibit complementary therapeutic effects against diseases. HRMS is now considered as a useful tool for the identification of these phytoconstituents in plants and has opened the possibility to provide more data in a time-efficient manner. Conjunction of HRMS with related techniques and methods can generate useful structural information for discovery and exploration of chemical constituents from plants. In this review, we have demonstrated the importance of HRMS in the characterization of natural products from plants; besides, the combination of HRMS with different separation techniques and screening strategies [113] can help identifying phytoconstituents from plants, which will increase the success rate in the discovery of new drugs and natural medicines. Some interesting trends in this hot area of research include the development of nanomaterials as non-interfering matrix for MALDI-MS analysis of small natural compounds [114], the use of metabolomics approaches to both identify new natural compounds and their possible bioactivity [115] as well as the development of specific software that enables the dereplication of previously identified metabolites and helps the discovery of structurally novel metabolites [116].

On the other hand, the performance of natural product screening programs still remains limited by the equipment available, labor and time investments required for the isolation and structural elucidation of novel metabolites with potent biological properties. Significant advances have been made over the last years to improve the resolution and sensitivity of analytical instruments and methods for the study of molecules in natural extracts or directly in the plant, for instance using methods based on mass spectrometry imaging (MSI) [117], a developing technique that

allows measuring the spatio-temporal distribution of many biomolecules in tissues. MSI can have an important impact in a broad range of areas, including primary metabolism, natural products, plant defense, plant responses to abiotic and biotic stress, plant lipids and the developing field of spatial metabolomics.

Also, very efficient hyphenation systems between separation and spectroscopic instruments now enable the identification of highly complex molecular structures even within complex mixtures of compounds. Despite these efforts, serious issues such as the frequent rediscovery of known compounds after time consuming purification and identification steps, or the investigation of irrelevant crude materials regarding a selected biological application, still results in a considerable waste of time. Consequently, many pharmaceutical companies have noticeably slowed down or even terminated their natural product research activities, indicating that there is still a long way to go in the improvement of the characteristics of the HRMS instruments and in the capabilities of the analytical strategies developed so far for natural products characterization.

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699 6. References

- 700 [1] P.K. Mukherjee, S. Bahadur, R.K. Harwansh, S. Biswas, S. Banerjee, Paradigm shift
701 in natural product research: traditional medicine inspired approaches, *Phytochemistry*
702 *Reviews* 16 (2017) 803-826.
- 703 [2] P. Kachlicki, A. Piasecka, M. Stobiecki, L. Marczak, Structural Characterization of
704 Flavonoid Glycoconjugates and Their Derivatives with Mass Spectrometric Techniques,
705 *Molecules* 21 (2016) 1494-1514.
- 706 [3] R.C.L. Lima, S.M. Gramsbergen, J. Van Staden, A.K. Jager, K.T. Kongstad, D.
707 Staerk, Advancing HPLC-PDA-HRMS-SPE-NMR Analysis of Coumarins in *Coleonema*
708 *album* by Use of Orthogonal Reversed-Phase C18 and Pentafluorophenyl Separations, *J Nat*
709 *Prod* 80 (2017) 1020-1027.
- 710 [4] J.-L. Wolfender, G. Marti, A. Thomas, S. Bertrand, Current approaches and
711 challenges for the metabolite profiling of complex natural extracts, *Journal of*
712 *Chromatography A* 1382 (2015) 136-164.
- 713 [5] A. Zhang, H. Sun, G. Yan, X. Wang, Recent developments and emerging trends of
714 mass spectrometry for herbal ingredients analysis, *TrAC Trends in Analytical Chemistry* 94
715 (2017) 70-76.
- 716 [6] A. Zhang, H. Sun, X. Wang, Mass spectrometry-driven drug discovery for
717 development of herbal medicine, *Mass Spectrom Rev* 37 (2018) 307-320.
- 718 [7] M.Z. Zhu, G.L. Chen, J.L. Wu, N. Li, Z.H. Liu, M.Q. Guo, Recent development in
719 mass spectrometry and its hyphenated techniques for the analysis of medicinal plants,
720 *Phytochem Anal* 29 (2018) 365-374.
- 721 [8] E. Gemperline, C. Keller, L. Li, Mass Spectrometry in Plant-omics, *Analytical*
722 *Chemistry* 88 (2016) 3422-3434.
- 723 [9] D. Pasin, A. Cawley, S. Bidny, S. Fu, Current applications of high-resolution mass
724 spectrometry for the analysis of new psychoactive substances: a critical review, *Anal Bioanal*
725 *Chem* 409 (2017) 5821-5836.
- 726 [10] H.Z. Senyuva, V. Gökmen, E.A. Sarikaya, Future perspectives in Orbitrap™ -high-
727 resolution mass spectrometry in food analysis: a review, *Food Additives & Contaminants:*
728 *Part A* 32 (2015) 1568-1606.
- 729 [11] L. Wang, Y. Liu, Y. Luo, K. Huang, Z. Wu, Quickly Screening for Potential alpha-
730 Glucosidase Inhibitors from Guava Leaves Tea by Bioaffinity Ultrafiltration Coupled with
731 HPLC-ESI-TOF/MS Method, *J Agric Food Chem* 66 (2018) 1576-1582.
- 732 [12] K. Iqbal, J. Iqbal, D. Staerk, K.T. Kongstad, Characterization of Antileishmanial
733 Compounds from *Lawsonia inermis* L. Leaves Using Semi-High Resolution Antileishmanial
734 Profiling Combined with HPLC-HRMS-SPE-NMR, *Front Pharmacol* 8 (2017) 337-343.
- 735 [13] E. Riethmuller, G. Toth, A. Alberti, K. Vegh, I. Burlini, A. Konczol, G.T. Balogh, A.
736 Kery, First characterisation of flavonoid- and diarylheptanoid-type antioxidant phenolics in
737 *Corylus maxima* by HPLC-DAD-ESI-MS, *J Pharm Biomed Anal* 107 (2015) 159-167.
- 738 [14] D. Ballesteros-Vivas, G. Álvarez-Rivera, E. Ibáñez, F. Parada-Alfonso, A. Cifuentes,
739 A multi-analytical platform based on pressurized-liquid extraction, in vitro assays and liquid
740 chromatography/gas chromatography coupled to high resolution mass spectrometry for food
741 by-products valorisation. Part 2: Characterization of bioactive compounds from goldenberry
742 (*Physalis peruviana* L.) calyx extracts using hyphenated techniques, *Journal of*
743 *Chromatography A* 1584 (2019) 144-154.
- 744 [15] M. Abbas-Mohammadi, M. Moridi Farimani, P. Salehi, S. Nejad Ebrahimi, A.
745 Sonboli, C. Kelso, D. Skropeta, Acetylcholinesterase-inhibitory activity of Iranian plants:
746 Combined HPLC/bioassay-guided fractionation, molecular networking and docking

747 strategies for the dereplication of active compounds, *J Pharm Biomed Anal* 158 (2018) 471-
748 479.

749 [16] R. Tong, M. Peng, C. Tong, K. Guo, S. Shi, Online extraction-high performance
750 liquid chromatography-diode array detector-quadrupole time-of-flight tandem mass
751 spectrometry for rapid flavonoid profiling of *Fructus aurantii immaturus*, *J Chromatogr B*
752 *Analyt Technol Biomed Life Sci* 1077-1078 (2018) 1-6.

753 [17] L.-L. Du, Y. Xue, J. Xie, Precursor Ion-Scan Mode-Based Screening Strategy for
754 New Amorfrutin Derivatives from *Amorpha fruticosa* by UPLC-QqQ-MS and UPLC-Q-
755 TOF-MS, *Chromatographia* 81 (2018) 769-776.

756 [18] J. Zhu, X. Yi, J. Zhang, S. Chen, Y. Wu, Rapid screening of brain-penetrable
757 antioxidants from natural products by blood-brain barrier specific permeability assay
758 combined with DPPH recognition, *Journal of Pharmaceutical and Biomedical Analysis* 151
759 (2018) 42-48.

760 [19] M. Zhou, X. Ma, J. Sun, G. Ding, Q. Cui, Y. Miao, Y. Hou, M. Jiang, G. Bai, Active
761 fragments-guided drug discovery and design of selective tropane alkaloids using ultra-high
762 performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry
763 coupled with virtual calculation and biological evaluation, *Anal Bioanal Chem* 409 (2017)
764 1145-1157.

765 [20] A. González, K. Wrobel, E. Yañez, A. Corrales Escobosa, J. Gutiérrez-Corona, I.
766 Enciso Donis, K. Wrobel, Changes of metabolomic profile in *helianthus annuus* under
767 exposure to Chromium(VI) Studied by capHPLC-ESI-QTOF-MS and MS/MS, 2017 (2017)
768 1-18.

769 [21] J. Zhu, X. Yi, W. Liu, Y. Xu, S. Chen, Y. Wu, Immobilized fusion protein affinity
770 chromatography combined with HPLC-ESI-Q-TOF-MS/MS for rapid screening of
771 PPAR γ ligands from natural products, *Talanta* 165 (2017) 508-515.

772 [22] R. Nakata, N. Yoshinaga, M. Teraishi, Y. Okumoto, A. Huffaker, E.A. Schmelz, N.
773 Mori, A fragmentation study of isoflavones by IT-TOF-MS using biosynthesized isotopes,
774 *Bioscience, Biotechnology, and Biochemistry* 82 (2018) 1309-1315.

775 [23] J.-G. Zhang, C.-A. Geng, X.-Y. Huang, X.-L. Chen, Y.-B. Ma, X.-M. Zhang, J.-J.
776 Chen, Chemical and biological comparison of different sections of *Uncaria rhynchophylla*
777 (Gou-Teng), *European Journal of Mass Spectrometry* 23 (2017) 11-21.

778 [24] G.-J. Wang, H.-X. Fu, J.-C. Xiao, W. Ye, T. Rao, Y.-H. Shao, D. Kang, L. Xie, Y.
779 Liang, Appropriate choice of collision-induced dissociation energy for qualitative analysis of
780 notoginsenosides based on liquid chromatography hybrid ion trap time-of-flight mass
781 spectrometry, *Chinese Journal of Natural Medicines* 14 (2016) 278-285.

782 [25] A. Angelis, L. Antoniadis, P. Stathopoulos, M. Halabalaki, L.A. Skaltsounis,
783 Oleocanthalic and Oleaceinic acids: New compounds from Extra Virgin Olive Oil (EVOO),
784 *Phytochemistry Letters* 26 (2018) 190-194.

785 [26] M.-E. Sakavitsi, M.-I. Christodoulou, J. Tchoumtchoua, N. Fokialakis, I.K.
786 Kokkinopoulou, E. Papageorgiou, A. Argyropoulou, L.A. Skaltsounis, M. Halabalaki, A.
787 Scorilas, Comparative HPLC-DAD and UHPLC-ESI(-)-HRMS & MS/MS profiling of
788 *Hypericum* species and correlation with necrotic cell-death activity in human leukemic cells,
789 *Phytochemistry Letters* 20 (2017) 481-490.

790 [27] J. Chervin, P. Perio, N. Martins-Froment, C. Pharkeovilay, K. Reybier, F. Nepveu, N.
791 Fabre, T. Talou, V. Bonzon-Ponnet, G. Marti, Dereplication of natural products from
792 complex extracts by regression analysis and molecular networking: case study of redox-
793 active compounds from *Viola alba* subsp. *dehnhardtii*, *Metabolomics* 13 (2017) 96-108.

794 [28] Y.J. Park, S.H. Seong, M.S. Kim, S.W. Seo, M.R. Kim, H.S. Kim, High-throughput
795 detection of antioxidants in mulberry fruit using correlations between high-resolution mass
796 and activity profiles of chromatographic fractions, *Plant Methods* 13 (2017) 108-120.

- 797 [29] S.T. Tasnuva, F. Sahena, U.A. Qamar, K. Ghafoor, J. Juliana, M.Z. Sarker, *Mimosa*
798 *pudica* L.: A comparative study via in vitro analysis and GC Q-TOF MS profiling on
799 conventional and supercritical fluid extraction using food grade ethanol, 8 (2017) 54-62.
- 800 [30] F. Carnevale Neto, A.C. Pilon, D.M. Selegato, R.T. Freire, H. Gu, D. Raftery, N.P.
801 Lopes, I. Castro-Gamboa, Dereplication of Natural Products Using GC-TOF Mass
802 Spectrometry: Improved Metabolite Identification by Spectral Deconvolution Ratio Analysis,
803 *Front Mol Biosci* 3 (2016) 59-71.
- 804 [31] O. Johan Juvik, B. Holmelid, G. Francis, H. Andersen, A. Paula de Oliveira, R.
805 Oliveira Junior, J. Guedes da Silva Almeida, T. Fossen, Non-Polar Natural Products from
806 *Bromelia laciniosa*, *Neoglaziovia variegata* and *Encholirium spectabile* (Bromeliaceae),
807 *Molecules* 22 (2017) 1478-1490.
- 808 [32] J. Peng, Y. Zhao, L. Xu, L.-p. Kang, J.-m. Cui, H.-s. Yu, J. Zhang, B.-p. Ma,
809 Metabolite profiling of steroidal glycosides in the rhizome of *Aspidistra sichuanensis* using
810 UPLC/Q-TOF MSE, *International Journal of Mass Spectrometry* 415 (2017) 63-84.
- 811 [33] Y. Du, Z.G. Zheng, Y. Yu, Z.T. Wu, D. Liang, P. Li, Y. Jiang, H.J. Li, Rapid
812 discovery of cyclopamine analogs from *Fritillaria* and *Veratrum* plants using LC-Q-TOF-MS
813 and LC-QqQ-MS, *J Pharm Biomed Anal* 142 (2017) 201-209.
- 814 [34] H. Zhao, X. Hu, X. Chen, S. Shi, X. Jiang, X. Liang, W. Chen, S. Zhang, Analysis
815 and improved characterization of minor antioxidants from leaves of *Malus doumeri* using a
816 combination of major constituents' knockout with high-performance liquid chromatography-
817 diode array detector-quadrupole time-of-flight tandem mass spectrometry, *J Chromatogr A*
818 1398 (2015) 57-65.
- 819 [35] S.H. Zhang, X. Hu, S.Y. Shi, L.Q. Huang, W. Chen, L. Chen, P. Cai, Typical
820 ultraviolet spectra in combination with diagnostic mass fragmentation analysis for the rapid
821 and comprehensive profiling of chlorogenic acids in the buds of *Lonicera macranthoides*,
822 *Anal Bioanal Chem* 408 (2016) 3659-3672.
- 823 [36] D. Li, T. Wang, Y. Guo, Y. Hu, B. Yu, J. Qi, Online screening of nitric oxide
824 scavengers in natural products using high performance liquid chromatography coupled with
825 tandem diode array and fluorescence detection, *J Chromatogr A* 1425 (2015) 106-115.
- 826 [37] H.P. Song, S.Q. Wu, L.W. Qi, F. Long, L.F. Jiang, K. Liu, H. Zeng, Z.M. Xu, P. Li,
827 H. Yang, A strategy for screening active lead compounds and functional compound
828 combinations from herbal medicines based on pharmacophore filtering and knockout/knockin
829 chromatography, *J Chromatogr A* 1456 (2016) 176-186.
- 830 [38] Y. Jiang, F.J. Liu, Y.M. Wang, H.J. Li, Dereplication-guided isolation of novel
831 hepatoprotective triterpenoid saponins from *Celosia* Semen by high-performance liquid
832 chromatography coupled with electrospray ionization tandem quadrupole-time-of-flight mass
833 spectrometry, *J Pharm Biomed Anal* 132 (2017) 148-155.
- 834 [39] J. Yuan, Y. Chen, J. Liang, C.Z. Wang, X. Liu, Z. Yan, Y. Tang, J. Li, C.S. Yuan,
835 Component analysis and target cell-based neuroactivity screening of *Panax ginseng* by ultra-
836 performance liquid chromatography coupled with quadrupole-time-of-flight mass
837 spectrometry, *J Chromatogr B Analyt Technol Biomed Life Sci* 1038 (2016) 1-11.
- 838 [40] Y. Wu, Y. Peng, C. Song, L. Li, H. Ma, D. Li, F. Wang, J. Yang, S. Song, C. Wu,
839 Separation and identification of multiple constituents in Xiao Chai Hu Decoction (Sho-saiko-
840 to) by bioactivity-guided fractionation combined with LC-ESI-QTOFMS/MS, *Biomed*
841 *Chromatogr* 29 (2015) 1146-1166.
- 842 [41] M. Zhao, M.M. Onakpa, W.-L. Chen, B.D. Santarsiero, S.M. Swanson, J.E. Burdette,
843 I.U. Asuzu, C.-T. Che, 17-Norpimaranes and (9 β H)-17-Norpimaranes from the Tuber of
844 *Icacina trichantha*, *Journal of natural products* 78 (2015) 789-796.
- 845 [42] S. Ji, D.D. He, T.Y. Wang, J. Han, Z. Li, Y. Du, J.H. Zou, M.Z. Guo, D.Q. Tang,
846 Separation and characterization of chemical constituents in *Ginkgo biloba* extract by off-line

hydrophilic interaction reversed-phase two-dimensional liquid chromatography coupled with quadrupole-time of flight mass spectrometry, *J Pharm Biomed Anal* 146 (2017) 68-78.

[43] I. Spanik, A. Machynakova, Recent applications of gas chromatography with high-resolution mass spectrometry, *J Sep Sci* 41 (2018) 163-179.

[44] T. Kind, H. Tsugawa, T. Cajka, Y. Ma, Z. Lai, S.S. Mehta, G. Wohlgemuth, D.K. Barupal, M.R. Showalter, M. Arita, O. Fiehn, Identification of small molecules using accurate mass MS/MS search, *Mass Spectrom Rev* 37 (2018) 513-532.

[45] R.A. Zubarev, A. Makarov, Orbitrap Mass Spectrometry, *Analytical Chemistry* 85 (2013) 5288-5296.

[46] L. Sun, G. Zhu, N.J. Dovichi, Comparison of the LTQ-Orbitrap Velos and the Q-Exactive for proteomic analysis of 1-1000 ng RAW 264.7 cell lysate digests, *Rapid Commun Mass Spectrom* 27 (2013) 157-162.

[47] N. Aligiannis, M. Halabalaki, E. Chaita, E. Kouloura, A. Argyropoulou, d. Ben, E. Kalpoutzakis, A. Angelis, K. Stathopoulou, S. Antoniou, M. Sani, V. Krauth, O. Werz, B. Schütz, H. Schäfer, M. Spraul, E. Mikros, L. A. Skaltsounis, Heterocovariance Based Metabolomics as a Powerful Tool Accelerating Bioactive Natural Product Identification, *Chemistry Select* 1 (2016) 2531-2535.

[48] H. Pan, W. Yang, Y. Zhang, M. Yang, R. Feng, W. Wu, D. Guo, An integrated strategy for the systematic characterization and discovery of new indole alkaloids from *Uncaria rhynchophylla* by UHPLC/DAD/LTQ-Orbitrap-MS, *Analytical and Bioanalytical Chemistry* 407 (2015) 6057-6070.

[49] K. Vougiotiannopoulou, M.T. Angelopoulou, H. Pratsinis, R. Grougnet, M. Halabalaki, D. Kletsas, B. Deguin, L.A. Skaltsounis, Chemical and Biological Investigation of Olive Mill Waste Water - OMWW Secoiridoid Lactones, *Planta Med* 81 (2015) 1205-1212.

[50] S.G. Leitão, G.G. Leitão, D.K.T. Vicco, J.P.B. Pereira, G. de Moraes Simão, D.R. Oliveira, R. Celano, L. Campone, A.L. Piccinelli, L. Rastrelli, Counter-current chromatography with off-line detection by ultra high performance liquid chromatography/high resolution mass spectrometry in the study of the phenolic profile of *Lippia origanoides*, *Journal of Chromatography A* 1520 (2017) 83-90.

[51] M.F. Tala, F.M. Talontsi, G.Z. Zeng, H.K. Wabo, M. Spiteller, N.H. Tan, P. Tane, Phytochemical Analysis of a Cytotoxic Fraction of *Quassia silvestris* using LC-HR-ESI-MS(n), *Phytochem Anal* 28 (2017) 210-216.

[52] A.C. Zanatta, A. Mari, M. Masullo, I. Zeppone Carlos, W. Vilegas, S. Piacente, L. Campaner Dos Santos, Chemical metabolome assay by high-resolution Orbitrap mass spectrometry and assessment of associated antitumoral activity of *Actinocephalus divaricatus*, *Rapid Commun Mass Spectrom* 32 (2018) 241-250.

[53] X. Qiao, R. Li, W. Song, W.J. Miao, J. Liu, H.B. Chen, D.A. Guo, M. Ye, A targeted strategy to analyze untargeted mass spectral data: Rapid chemical profiling of *Scutellaria baicalensis* using ultra-high performance liquid chromatography coupled with hybrid quadrupole orbitrap mass spectrometry and key ion filtering, *J Chromatogr A* 1441 (2016) 83-95.

[54] T. Nardin, E. Piasentier, C. Barnaba, R. Larcher, Targeted and untargeted profiling of alkaloids in herbal extracts using online solid-phase extraction and high-resolution mass spectrometry (Q-Orbitrap), *J Mass Spectrom* 51 (2016) 729-741.

[55] A. Jimenez-Gonzalez, C. Quispe, J. Borquez, B. Sepulveda, F. Riveros, C. Areche, E. Nagles, O. Garcia-Beltran, M.J. Simirgiotis, UHPLC-ESI-ORBITRAP-MS analysis of the native Mapuche medicinal plant palo negro (*Leptocarpha rivularis* DC. - Asteraceae) and evaluation of its antioxidant and cholinesterase inhibitory properties, *J Enzyme Inhib Med Chem* 33 (2018) 936-944.

- 897 [56] D.A. Chagas-Paula, T. Zhang, F.B. Da Costa, R. Edrada-Ebel, A Metabolomic
898 Approach to Target Compounds from the Asteraceae Family for Dual COX and LOX
899 Inhibition, *Metabolites* 5 (2015) 404-430.
- 900 [57] M.E. Gallon, O.A. Jaiyesimi, L. Gobbo-Neto, LC-UV-HRMS dereplication of
901 secondary metabolites from Brazilian Vernoniae (Asteraceae) species supported through in-
902 house database, *Biochemical Systematics and Ecology* 78 (2018) 5-16.
- 903 [58] A.M. Moricz, P.G. Ott, I. Yuce, A. Darcsi, S. Beni, G.E. Morlock, Effect-directed
904 analysis via hyphenated high-performance thin-layer chromatography for bioanalytical
905 profiling of sunflower leaves, *J Chromatogr A* 1533 (2018) 213-220.
- 906 [59] K.O. Eyong, G. Bairy, A.A. Eno, J. Taube, K.G. Hull, G.N. Folefoc, H.S. Foyet, D.
907 Romo, Triterpenoids from the stem bark of *Vitellaria paradoxa* (Sapotaceae) and derived
908 esters exhibit cytotoxicity against a breast cancer cell line, *Medicinal Chemistry Research* 27
909 (2018) 268-277.
- 910 [60] A.L. Capriotti, C. Cavaliere, G. La Barbera, C.M. Montone, S. Piovesana, R. Zenezini
911 Chiozzi, A. Lagana, Chromatographic column evaluation for the untargeted profiling of
912 glucosinolates in cauliflower by means of ultra-high performance liquid chromatography
913 coupled to high resolution mass spectrometry, *Talanta* 179 (2018) 792-802.
- 914 [61] E.-M. Pferschy-Wenzig, K. Koskinen, C. Moissl-Eichinger, R. Bauer, A Combined
915 LC-MS Metabolomics- and 16S rRNA Sequencing Platform to Assess Interactions between
916 Herbal Medicinal Products and Human Gut Bacteria in Vitro: a Pilot Study on Willow Bark
917 Extract, *Frontiers in Pharmacology* 8 (2017).
- 918 [62] Y. Bao, Y. Qu, J. Li, Y. Li, X. Ren, K.G. Maffucci, R. Li, Z. Wang, R. Zeng, In Vitro
919 and In Vivo Antioxidant Activities of the Flowers and Leaves from *Paeonia rockii* and
920 Identification of Their Antioxidant Constituents by UHPLC-ESI-HRMS(n) via Pre-Column
921 DPPH Reaction, *Molecules* 23 (2018) 392-406.
- 922 [63] D. Zheleva-Dimitrova, G. Zengin, V. Balabanova, Y. Voynikov, V. Lozanov, I.
923 Lazarova, R. Gevrenova, Chemical characterization with in vitro biological activities of
924 *Gypsophila* species, *Journal of Pharmaceutical and Biomedical Analysis* 155 (2018) 56-69.
- 925 [64] J.H. Gross, in: J.H. Gross (Ed.), *Matrix-Assisted Laser Desorption/Ionization*,
926 Springer International Publishing. 2017, pp. 651-720.
- 927 [65] R. Silva, N.P. Lopes, D.B. Silva, Application of MALDI Mass Spectrometry in
928 Natural Products Analysis, *Planta Medica* 82 (2016) 671-689.
- 929 [66] D.J. Harvey, Analysis of carbohydrates and glycoconjugates by matrix-assisted laser
930 desorption/ionization mass spectrometry: An update for 2013–2014, *Mass Spectrometry*
931 *Reviews* 37 (2018) 353-491.
- 932 [67] P. De Brum Vieira, N.L.F. Silva, C.B. Menezes, M.V. Da Silva, D.B. Silva, N.P.
933 Lopes, A.J. Macedo, J. Bastida, T. Tasca, Trichomonocidal and parasite membrane damaging
934 activity of bidesmosic saponins from *Manilkara rufula*, *PLoS ONE* 12 (2017) 1-20.
- 935 [68] D.P. Demarque, D.R. Callejon, G.G. de Oliveira, D.B. Silva, C.A. Carollo, N.P.
936 Lopes, The role of tannins as antiulcer agents: a fluorescence-imaging based study, *Brazilian*
937 *Journal of Pharmacognosy* 28 (2018) 425-432.
- 938 [69] S. Cibulski, M. Rivera-Patron, N. Suárez, M. Pirez, S. Rossi, A.C. Yendo, F. de
939 Costa, G. Gosmann, A. Fett-Neto, P.M. Roehe, F. Silveira, Leaf saponins of *Quillaja*
940 *brasiliensis* enhance long-term specific immune responses and promote dose-sparing effect in
941 BVDV experimental vaccines, *Vaccine* 36 (2018) 55-65.
- 942 [70] V.E. Cornelio, M.R. Forim, B. Perlatti, J.B. Fernandes, P.C. Vieira, M.P. Napolitano,
943 R.A. Yost, M.F.G.F. Da Silva, Identification of Meliatoxins in *Melia azedarach* Extracts
944 Using Mass Spectrometry for Quality Control, *Planta Medica* 83 (2017) 312-317.
- 945 [71] H.J. Hwang, I. Choi, Y.Y. Kang, H. Mok, Y. Lim, W.S. Yeo, Analysis of the
946 biodistribution of natural products in mice by using matrix-assisted laser

desorption/ionization time-of-flight mass spectrometry, *Applied Biological Chemistry* 61 (2018) 251-255.

[72] A. Altemimi, D.G. Watson, M. Kinsel, D.A. Lightfoot, Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a TLC-densitometric method, *Chemistry Central Journal* 9 (2015) 1-15.

[73] K. Kumar, B. Siva, N. Rama Rao, K. Suresh Babu, Rapid identification of limonoids from *Cipadessa baccifera* and *Xylocarpus granatum* using ESI-Q-ToF-MS/MS and their structure-fragmentation study, *Journal of Pharmaceutical and Biomedical Analysis* 152 (2018) 224-233.

[74] R.S. Phansalkar, J.W. Nam, S.N. Chen, J.B. McAlpine, J.G. Napolitano, A. Leme, C.M.P. Vidal, T. Aguiar, A.K. Bedran-Russo, G.F. Pauli, A galloylated dimeric proanthocyanidin from grape seed exhibits dentin biomodification potential, *Fitoterapia* 101 (2015) 169-178.

[75] J.M. Cui, L.P. Kang, Y. Zhao, J.Y. Zhao, J. Zhang, X. Pang, H.S. Yu, D.X. Jia, C. Liu, L.Y. Yu, B.P. Ma, Steroidal saponins from the rhizomes of *aspidistra typica*, *PLoS ONE* 11 (2016) 1-15.

[76] J. Hernandez, A. Müller, R. Jaiswal, J.Z. Davalos, N. Kuhnert, Energy resolved mass spectrometry of chlorogenic acids and its application to isomer quantification by direct infusion tandem mass spectrometry, *Phytochemical Analysis* 29 (2018) 406-412.

[77] B.Y. Yang, Z.L. Chen, Y. Liu, H.X. Kuang, Three new nortriterpenoids from the rattan stems of *Schisandra chinensis*, *Phytochemistry Letters* 24 (2018) 145-149.

[78] A. Natarajan, S. Sugumar, S. Bitragunta, N. Balasubramanyan, Molecular docking studies of (4Z, 12Z)-cyclopentadeca-4, 12-dienone from *Grewia hirsuta* with some targets related to type 2 diabetes, *BMC Complementary and Alternative Medicine* 15 (2015) 1-8.

[79] M. De Mieri, K. Du, M. Neuburger, P. Saxena, P.C. Zietsman, S. Hering, J.H. Van Der Westhuizen, M. Hamburger, hERG Channel Inhibitory Daphnane Diterpenoid Orthoesters and Polycephalones A and B with Unprecedented Skeletons from *Gnidia polycephala*, *Journal of Natural Products* 78 (2015) 1697-1707.

[80] M. De Mieri, G. Monteleone, I. Ismajili, M. Kaiser, M. Hamburger, Antiprotozoal Activity-Based Profiling of a Dichloromethane Extract from *Anthemis nobilis* Flowers, *Journal of Natural Products* 80 (2017) 459-470.

[81] D. Osei-Safo, G.A. Dziwornu, A. Salgado, S.N. Sunassee, M.A. Chama, Bi- and bisbibenzyls from the roots of *Dichapetalum heudelotii* and their antiproliferative activities, *Fitoterapia* 122 (2017) 95-100.

[82] M. Qiu, D. Cao, Y. Gao, S. Li, J. Zhu, B. Yang, L. Zhou, Y. Zhou, J. Jin, Z. Zhao, New clerodane diterpenoids from *Croton crassifolius*, *Fitoterapia* 108 (2016) 81-86.

[83] X. Zhao, X. Xi, Z. Hu, W. Wu, J. Zhang, Exploration of Novel Botanical Insecticide Leads: Synthesis and Insecticidal Activity of β -Dihydroagarofuran Derivatives, *Journal of Agricultural and Food Chemistry* 64 (2016) 1503-1508.

[84] S.R.M. Ibrahim, G.A. Mohamed, M.F. Zayed, S.A. Ross, 8-Hydroxyirilone 5-methyl ether and 8-hydroxyirilone, new antioxidant and α -amylase inhibitors isoflavonoids from *Iris germanica* rhizomes, *Bioorganic Chemistry* 61 (2014) 948-955.

[85] M. Korinek, V.D. Wagh, I.W. Lo, Y.M. Hsu, H.Y. Hsu, T.L. Hwang, Y.C. Wu, Y.B. Cheng, B.H. Chen, F.R. Chang, Antiallergic phorbol ester from the seeds of *Aquilaria malaccensis*, *International Journal of Molecular Sciences* 17 (2016) 398-410.

[86] S. Ji, Z. Li, W. Song, Y. Wang, W. Liang, K. Li, S. Tang, Q. Wang, X. Qiao, D. Zhou, S. Yu, M. Ye, Bioactive Constituents of *Glycyrrhiza uralensis* (Licorice): Discovery of the Effective Components of a Traditional Herbal Medicine, *Journal of Natural Products* 79 (2016) 281-292.

- 996 [87] Y. Dong, J. Liu, T. Guo, in: Y. Dong (Ed.), Introduction of Mass Spectrometry and
 997 Ambient Ionization Techniques, Wiley-VCH. Weinheim, Germany, 2017, pp. 1-42.
- 998 [88] D. Rondeau, in: Y. Dong (Ed.), DART Mass Spectrometry: Principle and Ionization
 999 Facilities, Wiley-VCH. Weinheim, Germany, 2017, pp. 43-80.
- 1000 [89] V. Bajpai, A. Singh, B. Kumar, K.P. Madhusudanan, in: Y. Dong (Ed.), Application
 1001 of DART-MS in Natural Phytochemical Research, Wiley-VCH. Weinheim, Germany, 2017,
 1002 pp. 255-290.
- 1003 [90] Y. Shen, W.-Y. Wu, D.-A. Guo, DART-MS: A New Research Tool for Herbal
 1004 Medicine Analysis, World Journal of Traditional Chinese Medicine 2 (2016) 2-9.
- 1005 [91] A. Singh, V. Bajpai, M. Srivastava, K.R. Arya, B. Kumar, Rapid screening and
 1006 distribution of bioactive compounds in different parts of *Berberis petiolaris* using direct
 1007 analysis in real time mass spectrometry, Journal of Pharmaceutical Analysis 5 (2015) 332-
 1008 335.
- 1009 [92] A. Rajchl, E. Fernández Cusimamani, J. Prchalová, R. Ševčík, H. Čížková, J.
 1010 Žiarovská, M. Hrdličková, Characterisation of yacon tuberous roots and leaves by DART-
 1011 TOF/MS, International Journal of Mass Spectrometry 424 (2018) 27-34.
- 1012 [93] P. Le Pogam, A.C. Le Lamer, B. Legouin, J. Boustie, D. Rondeau, In situ DART-MS
 1013 as a Versatile and Rapid Dereplication Tool in Lichenology: Chemical Fingerprinting of
 1014 *Ophioparma ventosa*, Phytochem Anal 27 (2016) 354-363.
- 1015 [94] S. Kumar, V. Bajpai, A. Singh, S. Bindu, M. Srivastava, K.B. Rameshkumar, B.
 1016 Kumar, Rapid fingerprinting of *Rauwolfia* species using direct analysis in real time mass
 1017 spectrometry combined with principal component analysis for their discrimination,
 1018 Analytical Methods 7 (2015) 6021-6026.
- 1019 [95] V. Bajpai, S. Kumar, A. Singh, N. Bano, M. Pathak, N. Kumar, S. Misra-
 1020 Bhattacharya, B. Kumar, Metabolic fingerprinting of dioecious *Tinospora cordifolia* (Thunb)
 1021 Miers stem using DART TOF MS and differential pharmacological efficacy of its male and
 1022 female plants, Industrial Crops and Products 101 (2017) 46-53.
- 1023 [96] J. Prchalova, F. Kovarik, A. Rajchl, Evaluation of the quality of herbal teas by
 1024 DART/TOF-MS, J Mass Spectrom 52 (2017) 116-126.
- 1025 [97] L. Habala, J. Valentova, I. Pechova, M. Fuknova, F. Devinsky, DART - LTQ
 1026 ORBITRAP as an expedient tool for the identification of synthetic cannabinoids, Legal
 1027 Medicine 20 (2016) 27-31.
- 1028 [98] B.L. Milman, I.K. Zhurkovich, Mass spectral libraries: A statistical review of the
 1029 visible use, TrAC Trends in Analytical Chemistry 80 (2016) 636-640.
- 1030 [99] B.L. Milman, General principles of identification by mass spectrometry, TrAC Trends
 1031 in Analytical Chemistry 69 (2015) 24-33.
- 1032 [100] M. Vinaixa, E.L. Schymanski, S. Neumann, M. Navarro, R.M. Salek, O. Yanes, Mass
 1033 spectral databases for LC/MS- and GC/MS-based metabolomics: State of the field and future
 1034 prospects, TrAC Trends in Analytical Chemistry 78 (2016) 23-35.
- 1035 [101] M. Wang, J.J. Carver, V.V. Phelan, L.M. Sanchez, N. Garg, Y. Peng, D.D. Nguyen, J.
 1036 Watrous, C.A. Kapon, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A.V. Melnik, M.J.
 1037 Meehan, W.-T. Liu, M. Crüsemann, P.D. Boudreau, E. Esquenazi, M. Sandoval-Calderón,
 1038 R.D. Kersten, L.A. Pace, R.A. Quinn, K.R. Duncan, C.-C. Hsu, D.J. Floros, R.G. Gavilan, K.
 1039 Kleigrew, T. Northen, R.J. Dutton, D. Parrot, E.E. Carlson, B. Aigle, C.F. Michelsen, L.
 1040 Jelsbak, C. Sohlenkamp, P. Pevzner, A. Edlund, J. McLean, J. Piel, B.T. Murphy, L.
 1041 Gerwick, C.-C. Liaw, Y.-L. Yang, H.-U. Humpf, M. Maansson, R.A. Keyzers, A.C. Sims,
 1042 A.R. Johnson, A.M. Sidebottom, B.E. Sedio, A. Klitgaard, C.B. Larson, C.A. Boya P, D.
 1043 Torres-Mendoza, D.J. Gonzalez, D.B. Silva, L.M. Marques, D.P. Demarque, E. Pociute, E.C.
 1044 O'Neill, E. Briand, E.J.N. Helfrich, E.A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H.
 1045 Mohimani, J.J. Kharbush, Y. Zeng, J.A. Vorholt, K.L. Kurita, P. Charusanti, K.L. McPhail,

1046 K.F. Nielsen, L. Vuong, M. Elfeki, M.F. Traxler, N. Engene, N. Koyama, O.B. Vining, R.
 1047 Baric, R.R. Silva, S.J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T. Hoffman, V.
 1048 Agarwal, P.G. Williams, J. Dai, R. Neupane, J. Gurr, A.M.C. Rodríguez, A. Lamsa, C.
 1049 Zhang, K. Dorrestein, B.M. Duggan, J. Almaliti, P.-M. Allard, P. Phapale, L.-F. Nothias, T.
 1050 Alexandrov, M. Litaudon, J.-L. Wolfender, J.E. Kyle, T.O. Metz, T. Peryea, D.-T. Nguyen,
 1051 D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K.M. Waters, W. Shi, X. Liu, L. Zhang, R.
 1052 Knight, P.R. Jensen, B.Ø. Palsson, K. Pogliano, R.G. Linington, M. Gutiérrez, N.P. Lopes,
 1053 W.H. Gerwick, B.S. Moore, P.C. Dorrestein, N. Bandeira, Sharing and community curation
 1054 of mass spectrometry data with Global Natural Products Social Molecular Networking,
 1055 Nature Biotechnology 34 (2016) 828-837.
 1056 [102] S.R. Johnson, B.M. Lange, Open-access metabolomics databases for natural product
 1057 research: present capabilities and future potential, Front Bioeng Biotechnol 3 (2015) 22-31.
 1058 [103] ChemSpider. <http://www.chemspider.com/>, 2019.
 1059 [104] C. Guijas, J.R. Montenegro-Burke, X. Domingo-Almenara, A. Palermo, B. Warth, G.
 1060 Hermann, G. Koellensperger, T. Huan, W. Uritboonthai, A.E. Aisporna, D.W. Wolan, M.E.
 1061 Spilker, H.P. Benton, G. Siuzdak, METLIN: A Technology Platform for Identifying Knowns
 1062 and Unknowns, Analytical Chemistry 90 (2018) 3156-3164.
 1063 [105] The Human Metabolome Database. <http://www.hmdb.ca/>, 2018.
 1064 [106] mzCloud Advanced Mass Spectral Database. <https://www.mzcloud.org/>, 2019.
 1065 [107] M. Chen, Y. Li, D. Xu, J. Luo, L. Kong, One-step targeted accumulation and
 1066 detection of camptothecin analogues from fruits of *Camptotheca acuminata* Decne using
 1067 bilayer solid-phase extraction coupled with ultra-high-performance liquid chromatography-
 1068 tandem mass spectrometry, J Chromatogr A 1524 (2017) 37-48.
 1069 [108] D. Ren, L. Ran, C. Yang, M. Xu, L. Yi, Integrated strategy for identifying minor
 1070 components in complex samples combining mass defect, diagnostic ions and neutral loss
 1071 information based on ultra-performance liquid chromatography-high resolution mass
 1072 spectrometry platform: *Folium Artemisiae Argyi* as a case study, J Chromatogr A 1550
 1073 (2018) 35-44.
 1074 [109] G.F. Feng, Y. Zheng, Y. Sun, S. Liu, Z.F. Pi, F.R. Song, Z.Q. Liu, A targeted strategy
 1075 for analyzing untargeted mass spectral data to identify lanostane-type triterpene acids in
 1076 *Poria cocos* by integrating a scientific information system and liquid chromatography-
 1077 tandem mass spectrometry combined with ion mobility spectrometry, Analytica Chimica
 1078 Acta 1033 (2018) 87-99.
 1079 [110] L.W. Sumner, A. Amberg, D. Barrett, M.H. Beale, R. Beger, C.A. Daykin, T.W.M.
 1080 Fan, O. Fiehn, R. Goodacre, J.L. Griffin, T. Hankemeier, N. Hardy, J. Harnly, R. Higashi, J.
 1081 Kopka, A.N. Lane, J.C. Lindon, P. Marriott, A.W. Nicholls, M.D. Reily, J.J. Thaden, M.R.
 1082 Viant, Proposed minimum reporting standards for chemical analysis Chemical Analysis
 1083 Working Group (CAWG) Metabolomics Standards Initiative (MSI), Metabolomics : Official
 1084 journal of the Metabolomic Society 3 (2007) 211-221.
 1085 [111] D.J. Creek, W.B. Dunn, O. Fiehn, J.L. Griffin, R.D. Hall, Z. Lei, R. Mistrik, S.
 1086 Neumann, E.L. Schymanski, L.W. Sumner, R. Trengove, J.-L. Wolfender, Metabolite
 1087 identification: are you sure? And how do your peers gauge your confidence?, Metabolomics
 1088 10 (2014) 350-353.
 1089 [112] L. Deng, A.M. Shi, H.Z. Liu, N. Meruva, L. Liu, H. Hu, Y. Yang, C. Huang, P. Li, Q.
 1090 Wang, Identification of chemical ingredients of peanut stems and leaves extracts using
 1091 UPLC-QTOF-MS coupled with novel informatics UNIFI platform, J Mass Spectrom 51
 1092 (2016) 1157-1167.
 1093 [113] F. Bucar, E. Wolfram, Bioassay-coupled Chromatography: Challenges and
 1094 Applications in Natural Product Research, 2017.

- [114] G. Han, Q. Zeng, Z. Jiang, T. Xing, C. Huang, Y. Li, MIL-101(Cr) as matrix for sensitive detection of quercetin by matrix-assisted laser desorption/ionization mass spectrometry, *Talanta* 164 (2017) 355-361.
- [115] C. Ma, L. Hu, X. Kou, W. Lv, Z. Lou, H. Wang, Rapid screening of potential α -amylase inhibitors from *Rhodiola rosea* by UPLC-DAD-TOF-MS/MS-based metabolomic method, *Journal of Functional Foods* 36 (2017) 144-149.
- [116] F. Qiu, D.D. Fine, D.J. Wherrett, Z. Lei, L.W. Sumner, PlantMAT: A Metabolomics Tool for Predicting the Specialized Metabolic Potential of a System and for Large-Scale Metabolite Identifications, *Analytical Chemistry* 88 (2016) 11373-11383.
- [117] B.A. Boughton, D. Thinakaran, D. Sarabia, A. Bacic, U. Roessner, Mass spectrometry imaging for plant biology: a review, *Phytochemistry Reviews* 15 (2016) 445-488.
- [118] Q. Gao, R. Ma, L. Chen, S. Shi, P. Cai, S. Zhang, H. Xiang, Antioxidant profiling of vine tea (*Ampelopsis grossedentata*): Off-line coupling heart-cutting HSCCC with HPLC-DAD-QTOF-MS/MS, *Food Chem* 225 (2017) 55-61.
- [119] X. Niu, J. Luo, D. Xu, H. Zou, L. Kong, Hydrogen/deuterium exchange, a unique and effective method for MS fragmentation behavior elucidation of ginkgolides and its application to systematic research in *Ginkgo biloba*, *J Pharm Biomed Anal* 134 (2017) 181-186.

Figure captions

Fig. 1 A) UPLC-DAD chromatogram (190 nm) and total ion chromatogram (TIC) in the positive mode of HSS; with the mass spectrum of three TA standards: D/L-anisodamine, scopolamine, and atropine. B) Fragmentation patterns of anisodamine and diagnostic ions of three TAs references. C) Extracted ion chromatogram (EIC) for m/z 122, 140, 158, 124, 156, 124, 142 based on the fragmentation patterns of the standards. Reprinted from [19] with permission of Elsevier.

Fig. 2 A) MALDI-TOF spectrum of a tannin-enriched fraction. B) Fragmentation pathway proposed to identify tannins. Reprinted from [68] with permission of Elsevier.

Fig. 3 Mass spectra in positive mode obtained by ESI-Q-TOF-MS of A) EtOAc extract of seeds of *Cipadessa baccifera* and B) EtOAc extract of fruits from *Xylocarpus granatum*. Reprinted from [73] with permission of Elsevier.

Fig 4 Workflow of the integrated filtering strategy proposed in [97]. NRF, nitrogen rule filtering; MDF, mass defect filtering; NLF, neutral loss filtering; DFIF, diagnostic fragment ion filtering; MFs, methoxylated flavonoids; CGAs, chlorogenic acids. Reprinted from [108] with permission of Elsevier.

Fig. 5 Integrated strategy for the discovery of new bioactive molecules from herbal medicines. Systematic characterization of indole alkaloids from UR as model study. Reprinted from [48] with permission of Elsevier.

Table 1. Selected stand-alone and hyphenated HRMS applications in the characterization of plant metabolites published in the period 2015-2018.

Secondary metabolites	Plan natural products	Hyphenated technique	Ion source	HRMS technique	Year	Ref.
Phenolic antioxidants	Mulberry fruit	Stand-alone	ESI	FT-ICR	2017	[28]
Phorbol ester	Aquilaria malaccensis	Stand-alone	ESI	FT-ICR	2016	[85]
Various (i.e. isoprenylated phenolics)	Glycyrrhiza uralensis	Stand-alone	ESI	FT-ICR	2016	[86]
Isoflavones	Agricultural products	HPLC	ESI	IT-TOF	2018	[22]
Alkaloids, flavonoids, triterpenoids, chlorogenic acid analogs	Uncaria rhynchophylla	UFLC-DAD	ESI	IT-TOF	2017	[23]
Notoginsenosides	Panax Notoginseng	HPLC	ESI	IT-TOF	2016	[24]
Norpimaranes	Icacina trichantha	LC	ESI	IT-TOF	2015	[41]
Galloylated dimeric proanthocyanidin	Grape seed	Stand-alone	ESI	IT-TOF	2015	[74]
Oleocanthalic and Oleaceinic acids	Virgin Olive Oil	UHPLC	ESI	LTQ-Orbitrap	2018	[25]
Various	Actinocephalus divaricatus	HPLC	ESI	LTQ-Orbitrap	2018	[52]
8-Hydroxyirilone 5-methyl ether and 8-hydroxyirilone isoflavonoids	Iris germanica rhizomes	Stand-alone	ESI	LTQ-Orbitrap	2017	[84]
Naphodianthrones, phloroglucinol derivatives and flavonoids	Hypericum species	UHPLC	ESI	LTQ-Orbitrap	2017	[26]
Phenolic compounds	Lippia origanoides	HPLC-DAD	ESI	LTQ-Orbitrap	2017	[50]
Redox-active compounds	Viola alba subsp dehnhardtii	UPLC-DAD	ESI	LTQ-Orbitrap	2017	[27]
Squalene-derived metabolites and oxasqualenoid derivatives	Quassia silvestris	HPLC	ESI	LTQ-Orbitrap	2017	[51]
β -Dihydroagarofuran derivatives	Celastrus angulatus Maxim	Stand-alone	ESI	LTQ-Orbitrap	2016	[83]
Various	Dorycnium, Cercis, Acronychia and Morus sp.	UHPLC	ESI	LTQ-Orbitrap	2016	[47]
Synthetic cannabinoids	Herbal materials (leaves, stems, flowers)	Stand-alone	DART	LTQ-Orbitrap	2016	[97]
Indole alkaloids	Uncaria rhynchophylla	UHPLC-DAD	ESI	LTQ-Orbitrap	2015	[48]
Secoiridoid Lactones	Olive Mill Waste Water	UHPLC	ESI	LTQ-Orbitrap	2015	[49]

Germacranolides, seco-sesquiterpenes, guaianolide sesquiterpene lactone, and cadinane acid	Anthemis nobilis Flowers	Stand-alone	ESI	microTOF	2017	[80]
Daphnane diterpenoid orthoesters and polycephalones A and B	Gnidia polycephala	Stand-alone	ESI	microTOF	2015	[79]
Various	Asteraceae Family	HPLC-UV	ESI	Q-Orbitrap	2015	[56]
Flavonoids	Gypsophila species	UHPLC	ESI	Q-Orbitrap	2018	[63]
Glucosinolates	Cauliflower	UHPLC	ESI	Q-Orbitrap	2018	[60]
(-)-Kaur-16-en-19-oic acid and 15- α -angeloyloxy-ent-kaur-16-en-19-oic acid diterpenes	Sunflower leaves	HPTLC	ESI	Q-Orbitrap	2018	[58]
Phenolic antioxidants	Flowers and Leaves from Paeonia rockii	UHPLC	ESI	Q-Orbitrap	2018	[62]
Hydroxycinnamic acid derivatives, flavonoids and sesquiterpene lactones.	Brazilian Vernoniaeae	UHPLC-DAD	ESI	Q-Orbitrap	2018	[57]
Triterpenoids and derived esters	stem bark of Vitellaria paradoxa	HPLC	ESI	Q-Orbitrap	2018	[59]
Sesquiterpenes, flavonoids, oxylipins, organic acids, and phenolic acids	Leptocarpha rivularis	UHPLC-DAD	ESI	Q-Orbitrap	2018	[55]
Phenolic compounds and glucoside derivatives	Willow Bark Extract	HPLC	ESI	Q-Orbitrap	2017	[61]
Flavones, flavanones, O-/C-glycosides, and phenylethanoid glycosides.	Scutellaria baicalensis	UHPLC	ESI	Q-Orbitrap	2016	[53]
Alkaloids	Herbal extracts	UHPLC	ESI	Q-Orbitrap	2016	[54]
Phenolic antioxidants, withanolides and sucrose esters	Physalis peruviana L. calyx	UHPLC/GC	ESI	QTOF	2018	[14]
Flavonoid and furanocoumarin type compounds.	Iranian plants (e.g. Prangos ferulacea)	HPLC	ESI	QTOF	2018	[15]
Gomisin H	Biodistribution in mice	Stand-alone	MALDI	QTOF	2018	[71]
Chlorogenic acids	Methanolic coffee extracts	Stand-alone	Direct infusion ER-MS	QTOF	2018	[76]
Flavonoids	Fructus aurantii immaturus	HPLC-DAD	ESI	QTOF	2018	[16]
Amorfrutin Derivatives	Amorpha fruticosa	UHPLC	ESI	QTOF	2018	[17]

Limonoids	<i>Cipadessa baccifera</i> and <i>Xylocarpus granatum</i>	Stand-alone	ESI	QTOF	2018	[73]
Phenolic antioxidants	Varios (e.g. <i>Rhizoma Anemarrhena</i>)	HPLC-UV	ESI	QTOF	2018	[18]
Tropane alkaloids	<i>Physochlaina infundibularis</i> Kuang	UHPLC-DAD	ESI	QTOF	2017	[19]
Flavonoids	<i>Ampelopsis grossedentata</i>	HPLC-DAD	ESI	QTOF	2017	[118]
Various	<i>Helianthus annuus</i>	capHPLC	ESI	QTOF	2017	[20]
Celosiae Semen	Triterpenoid saponins	HPLC	ESI	QTOF	2017	[38]
Phenolic antioxidants	Mulberry fruit	HPLC	ESI	QTOF/FT-ICR	2017	[28]
Ginkgolides	<i>Ginkgo biloba</i>	HPLC	ESI	QTOF	2017	[119]
PPAR γ ligands (e.g. Isochlorogenic acid A)	<i>Dendranthema indicum</i> flowers	HPLC	ESI	QTOF	2017	[21]
Steroidal glycosides	Rhizome of <i>Aspidistra sichuanensis</i>	UHPLC	ESI(+/-)	QTOF	2017	[32]
Various (e.g. resorcinol, oleic acid, and ethyl allocholates)	<i>Mimosa pudica</i> L.	GC	EI	QTOF	2017	[29]
Camptothecin analogues	<i>Camptotheca acuminata</i> Decne	UHPLC	ESI	QTOF	2017	[107]
Cyclopamine analogs	<i>Fritillaria</i> and <i>Veratrum</i> plants	HPLC	ESI	QTOF	2017	[33]
Phenolic compounds and glucoside derivatives	<i>Ginkgo biloba</i>	2D-HPLC-DAD	ESI	QTOF	2017	[42]
Various active lead compounds	Herbal medicine preparation (Dan-Qi formula, DQF)	HPLC	ESI	QTOF	2016	[37]
Gensenosides	<i>Panax ginseng</i>	HPLC	ESI	QTOF	2016	[39]
Steroidal Saponins	Rhizomes of <i>Aspidistra typica</i>	Stand-alone	ESI	QTOF	2016	[75]
Chlorogenic acids	<i>Lonicera macranthoides</i>	HPLC-DAD	ESI	QTOF	2016	[35]
Phenolic antioxidants	<i>Malus doumeri</i> leaves	HPLC-DAD	ESI	QTOF	2015	[34]
4Z, 12Z)-cyclopentadeca-4, 12-dienone	<i>Grewia hirsuta</i>	Stand-alone	ESI	QTOF	2015	[78]
Flavonoids and tanins	Various (e.g. <i>S. baicalensis</i> , green tea.)	HPLC	ESI	QTOF	2015	[36]

Flavonoids, saikosaponins, ginsenosides, licoricesaponins and gingerols	Xiao Chai Hu Decoction Chinese medicine formula	HPLC	ESI	QTOF	2015	[40]
Saponins	Quillaja brasiliensis	Stand-alone	MALDI	TOF	2018	[69]
Flavonoids and procyanidins	Guava leaves tea	HPLC	ESI	TOF	2018	[11]
Tanins	Stryphnodendron rotundifolium Mart.	Stand-alone	MALDI	TOF	2018	[68]
Nortriterpenoids	Rattan stems of Schisandra chinensis	Stand-alone	ESI	TOF	2018	[77]
Various	Yacon tuberous roots and leaves	Stand-alone	DART	TOF	2018	[92]
Various (e.g. 2,4,6-trihydroxyacetophenone-2-O- β -D-glucopyranoside, lalioside, luteolin-4'-O- β -D-glucopyranoside, apigenin-4'-O- β -D-glucopyranoside, luteolin, and apigenin)	Lawsonia inermis L. Leaves	HPLC	ESI	TOF	2017	[12]
Various non-polar metabolites (e.g. tocopherols, phytol, triterpenoid derivatives)	Bromelia laciniosa, Neoglaziovia variegata and Encholirium spectabile	GC	EI	TOF	2017	[31]
Glucoside flavonoids	Rhodiola rosea	UHPLC-DAD	ESI	TOF	2017	[115]
Various (e.g. N-Methyl-2-pyrrolidone, Arginine, 11-Hydroxymustakone, Tinocordifolin, N-Formylanonaine, N-trans-Feruloyltyramine, Reticuline, Jatrorrhizine, Magnoflorine, Palmatine)	Dioecious Tinospora cordifolia (Thunb) Miers	Stand-alone	DART	TOF	2017	[95]
Glycosides, flavonoids, phenolic and terpenic substances, such as chamazulene, anethole, menthol, thymol, salviol, hypericin	Herbal teas	Stand-alone	DART	TOF	2017	[96]
Dibenzofuran-related usnic acid, depsidones, naphthoquinone, depsides.	Ophioparma ventosa	Stand-alone	DART	TOF	2016	[93]
Flavonoid- and diarylheptanoid-type antioxidant phenolics	Corylus maxima	HPLC-DAD	ESI	TOF	2015	[13]
Flavonoids and polyphenols	Peach and pumpkin extracts	Stand-alone	MALDI	TOF	2015	[72]

Various (e.g. reticuline, thalifendine/berberrubine, demethyleneberberine, berberine, jatrorrhizine, tetrahydroberberine, magnoflorine)	<i>Berberis petiolaris</i>	Stand-alone	DART	TOF	2015	[91]
Various	<i>Rauwolfia</i> species	Stand-alone	DART	TOF	2015	[94]
Bidesmosic saponins	<i>Manilkara rufula</i>	Stand-alone	MALDI	TOF/TOF	2017	[67]

Figure 1.

Figure 2

Figure 3

A

B

Figure 4.

Figure 5