



ASAP-MS and DART-MS as ancillary tools for direct analysis of the lichen metabolome

Simon Ollivier, Philippe Jéhan, Fabian Lambert, Damien Olivier-jimenez, Joël Boustie, Françoise Lohézic-le Dévéhat, Nicolas Le Yondre

► To cite this version:

Simon Ollivier, Philippe Jéhan, Fabian Lambert, Damien Olivier-jimenez, Joël Boustie, et al.. ASAP-MS and DART-MS as ancillary tools for direct analysis of the lichen metabolome. *Phytochemical Analysis*, 2022, 33 (7), pp.1028-1035. 10.1002/pca.3156 . hal-03715437

HAL Id: hal-03715437

<https://hal.science/hal-03715437>






Submitted on 10 Jul 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RESEARCH ARTICLE

ASAP-MS and DART-MS as ancillary tools for direct analysis of the lichen metabolome

Simon Ollivier^{1,2}  | Philippe Jéhan¹ | Fabian Lambert¹ |
Damien Olivier-Jimenez²  | Joël Boustie²  | Françoise Lohézic-Le Dévéhat²  |
Nicolas Le Yondre¹ 

¹ScanMAT UAR 2025, CRMPO (Centre Régional de Mesures Physiques de l'Ouest), Univ Rennes, CNRS, Rennes, France

²ISCR (Institut des Sciences Chimiques de Rennes) – UMR 6226, Univ Rennes, CNRS, Rennes, France

Correspondence

Dr. Nicolas Le Yondre, Univ Rennes, CNRS, ScanMAT UAR 2025, CRMPO (Centre Régional de Mesures Physiques de l'Ouest), F-35000 Rennes, France.
Email: nicolas.leyondre@univ-rennes1.fr

Dr. Françoise Lohézic-Le Dévéhat, Univ Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes) – UMR 6226, F-35000 Rennes, France.
Email: francoise.le-devehat@univ-rennes1.fr

Present address

Simon Ollivier, INRAE, UR BIA, F-44300 Nantes, France and INRAE, PROBE research infrastructure, BIBS facility, F-44300 Nantes, France

Abstract

Introduction: Lichens contain unique metabolites that most often need to be characterized from a limited amount of material. While thin layer chromatography is still the preferred analysis method for most lichenologists, liquid chromatography gives a deeper insight in the lichen metabolome, but an extractive step is needed before any analysis. Therefore, ambient ionization mass spectrometry (MS) analysis of lichen samples using Atmospheric Solid Analysis Probe (ASAP) and Direct Acquisition in Real Time (DART) techniques is evaluated.

Objective: We looked for a faster method to screen the metabolome by disrupting the classical workflow of analysis.

Methods: Four lichens selected for their metabolic diversity were analyzed with MS; namely *Evernia prunastri*, *Lichina pygmaea*, *Parmelia saxatilis*, and *Roccella fuciformis*. ASAP and DART analyses were compared against the reference electrospray ionization with a bioinformatic process including Van Krevelen diagrams as well as the multivariate comparison of the ionization methods in positive and negative modes.

Results: Metabolite profiles obtained from DART and ASAP analyses of lichen samples are consistent with classical analyses of lichen extracts. Through an easy and rapid experiment and without any extraction solvent, a large and informative profile of lichen metabolites is obtained when using complementary ionization modes of these high resolution mass spectrometry methods.

Conclusion: ASAP-MS and DART-MS are two ancillary methods that provide a comprehensive evaluation of the lichen metabolome.

KEYWORDS

ionization techniques, lichens, mass spectrometry, specialized metabolites

SO, PJ, FLD, JB, and NLY designed the experiments. SO and FLD selected the lichen material, which was identified by JB. DO provided advice on the sample preparation and feedback on the results. SO, PJ, and FL carried out the experiments. All authors contributed to the redaction of the manuscript. None of the authors declare any competing financial interest.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Phytochemical Analysis* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Lichens are ubiquitous organisms spread all over the world even if they are understudied. The union of microscopic mycobiont and photobiont forms this symbiotic organism which may appear as crustose, foliaceous, squamulose, fruticose thalli. Colors of thalli vary from orange to white including black suggesting different colors of the main lichen metabolites. The chlorophyllous partner (green microalgae or cyanobacteria) notably synthesizes carbohydrates (glucose, mannitol, arabinol) thanks to the photosynthesis and the fungus can synthesize amino acids *de novo*. Three biogenetic pathways can be activated: the mevalonate pathway can give terpenoids, unsaturated hydrocarbons, fatty acyls, and prenyl derivatives; the acetyl-polymalonyl pathway results in phenolic compounds and benzenoids; and the condensed aromatic compound and shikimic pathways lead to condensed aromatic compounds and mycosporines.^{1,2} Sample size is often a setback for lichenologists aiming to establish a metabolic profile, and solvent extraction is by itself a bias—all solvents have different properties, and their choice highly impacts the observed profile.³ In some cases, solvent extraction even generates artifact compounds,⁴ bringing out the need for direct ionization methods for a rapid screening of the lichen metabolome. To this day, thin layer chromatography remains the main method used by lichenologists,⁵ therefore, the chemistry of lichens is rarely investigated up to trace level compounds leaving an untapped and potentially important source of novel chemical structures. Recent findings in molecular biology support this hypothesis, demonstrating a potential for biosynthesis that overshadows the number of known lichen specialized metabolites.⁶ A high resolution tandem mass spectrometry (MS/MS) spectral library for the dereplication of lichen compounds has recently been published as a powerful and versatile method, at the same time revealing a large number of unknown lichen compounds through liquid chromatography–high resolution MS (LC–HRMS) studies, bioinformatic processing for dereplication, and molecular network approaches.⁷ Such library allows to match the fragmentation spectra from complex mixtures with known standards, but the fragmentation can vary according to the ionization mode (i.e., adducts) and the mass spectrometer geometry. It is highly efficient for LC–MS analyses, but the bioinformatic processing can be quite heavy—requiring pre-processing⁸ and often crossing the results from the library to data from several other annotation tools (e.g., Refs^{9–12})—and it only brings limited information for unknown compounds.

It seems crucial to develop a rapid exploration tool to decrypt the chemical diversity of lichen metabolites profiled by HRMS methods. In this aspect, the performance of two ambient ionization methods, Atmospheric Solid Analysis Probe (ASAP) and Direct Analysis in Real Time (DART), is evaluated for use directly on lichen thalli. The basic principles of these two methods are well described with similarities and some important differences (for a review, see Gross 2014).¹³ Briefly, ASAP can be performed on a normal APCI ion source and ion formation is mainly initiated by corona discharge; as a result, molecular ions, protonated molecules, or deprotonated molecules are formed; DART is a glow discharge ionization method

and initiates a flow of gas containing metastable atoms (helium, typically) by the so-called Penning ionization mechanism. Ambient air (N₂, O₂, and H₂O) is ionized by this flow. The sample in front of the source receives the flow and ions are produced, mainly by reaction with the ionized ambient air. As a result, during a DART experiment, not only classical ions like molecular ions, protonated molecules, and deprotonated molecules are detected, but also different interesting [M + NH₄]⁺ adducts.

DART–MS is especially popular for the direct analysis of complex biological samples (e.g., botanical drugs, enzymatic substrates,¹⁴ or crude propolis¹⁵). This method has even been previously used directly on lichen thalli: DART–MS has notably been used for the chemical profiling of the lichen *Ophioparma ventosa* and for the thermochemical study of mycosporine serinol from *Lichina pygmaea*.^{16,17}

Four lichen species (*Evernia prunastri* (L.) Ach., *L. pygmaea* (Lightf.) C. Agardh., *Parmelia saxatilis* (L.) Ach., and *Roccella fuciformis* (L.) DC) were collected and the tool chosen to exhibit the results was the Van Krevelen (VK) diagram¹⁸ that sorts molecules depending on their elemental composition (according to their H/C and O/C ratios). It can be generated through molecular formula determination from HRMS and used for inferring gross structural information in metabolomics (for a review, see Rathahao-Paris et al.).¹⁹ Recently, Castilla et al.²⁰ showed that the VK diagram was also useful for the comparison of MS ion sources: they compared Direct Inlet Probe Atmospheric Pressure Photo and Chemical Ionization), respectively DIP–APPI and DIP–APCI, in a study about lignocellulosic biomass. Although this purpose is far from that of metabolomics, they interestingly showed that DIP–APCI ionized a greater variety of compounds than DIP–APPI (especially fatty acids).

In this article, the potential of raw material analysis with ambient ionization MS for the exploration of lichen metabolomes is exploited. The comparison of ASAP–MS and DART–MS raw material analyses with direct infusion (DI)–electrospray ionization (ESI)–MS experiments on extract material and an in-house database of around 2,000 lichen compounds (LDB–Lit)²¹ is proposed to give a deeper analytical insight. Through examples of ionization profiles—ranging from raw mass spectra to processed data—and multivariate analysis, the respective interests of ASAP–MS and DART–MS for lichen metabolomics will be shown.

2 | EXPERIMENTAL PROCEDURES

2.1 | General procedures

All solvents and reagents used in this study were HPLC grade (Sigma–Aldrich) and were used without further purification. The contamination by plastic compounds was limited through the use of glass containers. For experiments requiring the thermal desorption of ground lichens, Marenfeld melting point capillaries (100 × 1.5 mm, No. 29402 10, Paul Marienfeld GmbH & Co. KG, Lauda–Königshofen, Germany) were used to introduce the samples in the ASAP ion source.

2.2 | Lichen material

This study was conducted on herbarium specimens of *E. prunastri*, *L. pygmaea*, *P. saxatilis*, and *R. fuciformis*. They were collected in Cressensac, France (N 45°0'19.321" O 1°30'51.191") in August 2006 for *E. prunastri*; in Roscoff, France (N 48°43'31.458" O -3°58'8.651") in June 2016 for *L. pygmaea*; in Girona, Spain (N 42°27' 53.399" O 1°47'23.816") in July 2017 for *P. saxatilis*; and in Saint-Coulomb, France (N 48°41'29.299" O -1°56'45.855") in May 2003 for *R. fuciformis*. Vouchers are deposited in the herbarium of the University of Rennes, France under registration numbers JB/06/51, JB/16/204, JB/17/213, and JB/03/02, respectively.

2.3 | Sample preparation

For each lichen specimen: (1) An intact piece was preserved for on-axis DART-MS analysis; (2) raw lichen was ground in liquid nitrogen to avoid sample degradation; and (3) part of the lichen powder was extracted using successive cyclohexane-acetone-methanol (CAM) extractions. Briefly, the lichens were extracted using these three solvents successively. Then, the extracts were recombined *pro rata* to the extracted quantities to give CAM extracts. All extractions were done on an Accelerated Speed Extractor E-914 (ASE) (Büchi, Villebon Sur Yvette, France) after dispersion of ground lichens in celite (1:1 ratio, m/m). Three maceration cycles of 10 min in 40°C solvent with a discharge time of 2 min were applied for this extraction.

Briefly, stage (1) samples were analyzed with DART-MS, stage (2) samples were analyzed with ASAP-MS, and stage (3) samples as extracts were analyzed with DI-ESI-MS.

2.4 | HR-MS analyses

ASAP-MS and DART-MS analyses on raw lichen were compared with DI-ESI-MS and LDB-Lit. The DI-ESI-MS data of a CAM extract give the most comprehensive profile. Thus, the maximum level of information obtainable is reached from extracts by infusing samples in methanol.²²

DART-MS analyses were performed on a Q-Exactive mass spectrometer (hybrid quadrupole-orbitrap Q-OT technology; Thermo Scientific, Bremen, Germany) equipped with a Vapor DART SVP ion source, with a Rev 4 heating system (Ion Sense, Saugus, MA, US). ASAP-MS analyses were performed on a Maxis 4G spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an APCI ion source. Two biological replicates were performed for both DART-MS and ASAP-MS.

DI-ESI-MS analyses were performed on a Q-Exactive mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an Ion Max API source and an H-ESI II Electrospray probe (Thermo Scientific, Bremen, Germany). Three technical replicates in methanol as ionization solvent were performed for DI-ESI-MS.

For ASAP-MS analyses, ground lichens were deposited on melting point capillaries by dipping into the ground material, rubbing, and finally tapping on a glass surface to get as little matter as possible before insertion in the APCI source. Samples were analyzed as described for their extract counterparts with a temperature ramp (50°C every 15 sec) over 2 min within 50–400°C with 120 scans in the *m/z* range 100–2,000. More detailed parameters are given in **Supplementary text 1**. Data were acquired in single batch analysis with a thermal desorption to noise level between every sample, and controls showed no loss of sensitivity.

For DART-MS analyses, lichen thalli were maintained with a clamp directly in front of the ionization source. Samples were analyzed in the on-axis mode using helium (He Alphagaz 1, purity 99.999%, AirLiquide) as the desorption-ionization gas. Thus, with regard to the geometry of the ionization source and the gas pressure, ground lichens were not analyzed in order to avoid damaging the instrument. More detailed parameters are given in **Supplementary text 2**. Acquisition was done over 2 min (230 scans) in a source temperature range of 100–450°C with 50°C steps, and lock-mass calibration was applied on palmitic acid during acquisition (deprotonated molecule $[M-H]^-$ *m/z* 255.2330 and protonated molecule $[M+H]^+$ *m/z* 257.2475). No loss of sensitivity was observed across experiments.

For DI-ESI-MS analyses, lichen extracts were dissolved in methanol at a concentration of 15 µg/mL. The flowrate was 3 µL/min. More detailed parameters are given in **Supplementary text 3**.

2.5 | Bioinformatic data processing

Data were processed according to a classification approach based on the VK diagram.¹⁸ It was used as described with the scripts provided at <https://github.com/siollivier/directacquisitionproject>. Briefly, the scripts generate the best molecular formulae from HRMS data, apply an adduct correction, and then classify the features depending on elemental ratios. $[M+NH_4]^+$ adducts were additionally taken into account when generating the molecular formulae for DART-MS. The processed dataset, including the DI-ESI-MS analyses, was added to our OSF project for Ambient Ionization MS at <https://osf.io/6pyuq/> (dataset_pre-extract_comp_publi2.tsv).

2.6 | Multivariate data analysis

Both adduct-corrected and uncorrected peak lists were exported to *.csv files with R-script and analyzed with the online platform MetaBoAnalyst 5.0.²³ The adduct correction allowed direct comparison of the different techniques and of both polarities. The data were binned with a tolerance of $\pm 5.10^{-4}$ u (0.5 mu) and missing values were replaced by a small value. Data were also filtered with an interquartile range and then normalized by applying quantile normalization, log transformation, and Pareto scaling to limit the influence of peak intensity, as the data were acquired on two different instruments.

When our VK diagram-based protocol was applied for profiling purposes, the data of all lichens were merged. When showing spectra or statistical analyses, examples on a single lichen are provided to retain significance.

3 | RESULTS AND DISCUSSION

Since the object of this study is to find a way to explore the entirety of the metabolome without performing any extraction, it is primordial that the selected techniques allow for the desorption of the whole sample and not only of surface metabolites. Considering ASAP-MS and DART-MS use heat to desorb molecules, they appear ideal. The initial hypothesis is that, at the cost of a small amount of material, (1) these two techniques may be of use for a direct assessment of lichen metabolome and (2) combining these techniques should provide more insight into the species' metabolome.

3.1 | Mass spectrometry profiles of ASAP and DART

In order to compare ASAP-MS and DART-MS, the ionization profiles in the raw mass spectra are first considered. Indeed, only considering the output from a bioinformatic processing could easily induce mistakes. Figure 1 presents the spectra from *P. saxatilis*, which had the ionization profile that best illustrated this hypothesis (the differences between ASAP and DART are more obvious without having to zoom in on low intensity compounds). This species will be used throughout this article, for all analyses that must be exemplified. It is apparent that, in both ion modes, the relative intensities of the major peaks are widely different.

For example, in positive ion mode, the most intense peak of the DART spectrum is m/z 413 (exact mass 413.1956, $[M + H]^+$ molecular formula $C_{24}H_{28}O_6$), whereas it is m/z 395 (exact mass 395.3164, $[M + H]^+$ molecular formula $C_{24}H_{42}O_4$) in the ASAP spectrum. In negative ion mode, the most intense peak of the DART spectrum is m/z 455 (exact mass 455.1708 $[M-H]^-$, molecular formula $C_{24}H_{28}O_8$), whereas it is m/z 417 (exact mass 417.3234, $[M-H]^-$ molecular formula $C_{23}H_{46}O_6$) in the ASAP spectrum. Furthermore, in negative ion mode, DART-MS allows an intense detection of compounds over m/z 550.

Some differences appear in the low m/z range between ASAP and DART; this is mainly due to the strategies chosen to optimize detection across the whole m/z range. DART-MS data were obtained with a Q-OT, on which this optimization was done through the S-lens RF level, whereas for ASAP-MS, an ion-cooler RF voltage was applied in the Q-TOF instrument. This ion-cooler RF still allows the transmission of the ions, but lowers their intensity, making them less apparent on the spectra.

For the comparison of both techniques, the distribution of compounds across the entire m/z range was also studied: raw spectra are biased by intensity, whereas a density plot of all detected masses gives a more global insight (Figure 2). For the compounds detected by the combination of ASAP-MS and DART-MS, a good overlap is noted with the data obtained with classical DI-ESI-MS analysis of extracts. The global density of ASAP and DART is well fitted to the curb of DI-ESI for m/z ratios above 300; however, values below m/z 300 overfit ESI by a large margin, notably thanks to the contribution of DART-MS analyses (even though DART-MS was carried out on the same instrument as DI-ESI-MS). A quick observation of the LDB-Lit profile shows the interest of the project that could bring some new coordinates under m/z 300 and above m/z 600, after a complete proper dereplication process.

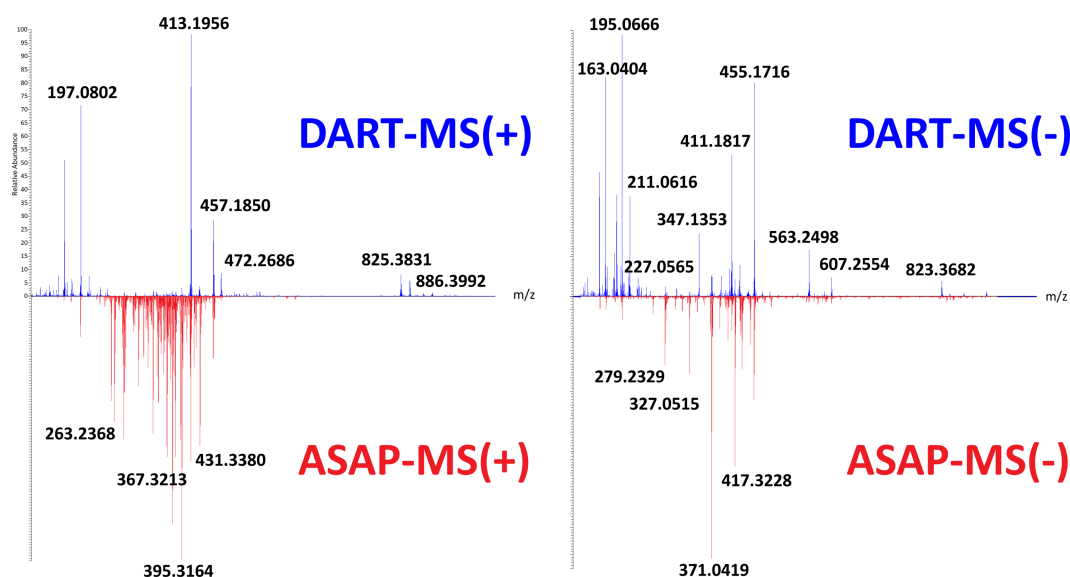


FIGURE 1 A mirror comparison of DART-MS (blue) and ASAP-MS (red) shows substantially different ionization profiles in both positive (left) and negative ion mode (right). The presented spectra—obtained from *Parmelia saxatilis*—are averaged over the whole temperature ramps. All associated data and annotations are available at <https://osf.io/6pyuq>

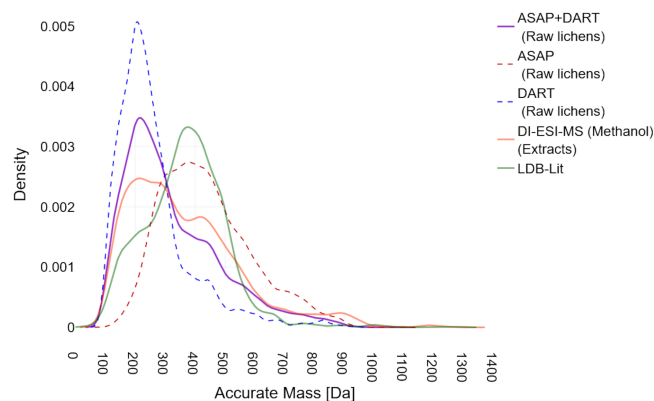


FIGURE 2 Distribution of the calculated exact masses obtained through the analysis of lichens by DI-ESI-MS after extraction (orange solid line), the combination of ASAP and DART-MS on raw lichen (purple solid line), ASAP-MS (red dotted line), and DART-MS (blue dotted line) as standalone means of analysis, and the distribution of an in-house database (LDB-lit) (green solid line)

3.2 | Direct ASAP-MS and DART-MS profiling of the lichen metabolome

Overall, the combination of ASAP and DART appeared interesting because they could potentially provide information for a wide range of compounds. So, the metabolome of four lichen species was profiled—*E. prunastri*, *L. pygmaea*, *P. saxatilis*, and *R. fuciformis*—to gain additional insight on the types of compounds ionized and a comparison was performed with DI-ESI-MS experiments on extracts and an in-house database of lichen compounds to prove the entirety of the analysis process and content (i.e., surface and internal metabolites and general knowledge).

In the present study, these features are similarly detected from MS analyses performed directly on the lichens. In positive ion mode, the ionization profile is similar for both techniques, and DART-MS detects more compounds (average number of ions 2,754/run vs. 1,211/run for ASAP-MS). In negative mode DART also complements ASAP-MS: it appears to alleviate a lack of detection of polyphenols and derivatives. DI-ESI-MS on the extracts represents the entirety in terms of detection capacity for surface and internal metabolites in ASAP-MS and DART-MS; the in-house database represents a milestone of lichen characterization knowledge. There are some specificities like the ionization of terpenes and unsaturated hydrocarbons in DART-MS(+) or ASAP-MS(+), of polyphenols and derivatives in DART-MS(–), or of condensed aromatic compounds in ASAP-MS(–), but all families are represented (Figure 3a and 3b, the numeric values are summarized in Table S1). Raw material analyses in both methods allow a detection in every chemical family, demonstrating their potential for screening purposes.

Although these profiles, filtered by chemical classes, show high similarity, a throwback to the VK diagram illustrates the complementarity of both techniques within a given chemical group

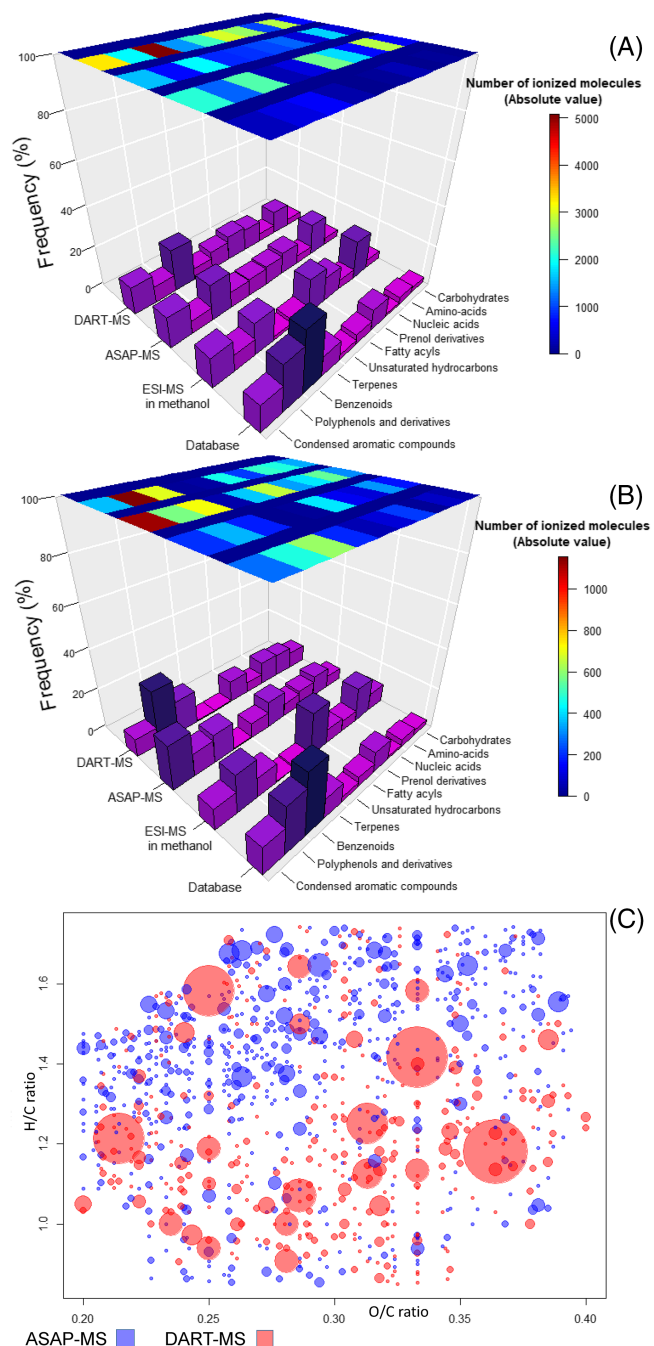


FIGURE 3 Evaluation of the chemical diversity of the raw lichen material compared with an in-house database (LDB-lit) in positive (A) and negative ionizations (B). Both the relative frequency within each technique (histogram) and the absolute number of detected ions (heatmap) are presented. (C) Extracted Van Krevelen diagram for benzenoids (or atypical terpenoids) in ASAP-MS and DART-MS. The size of the circle is proportional to the number of occurrences of the (O/C, H/C) coordinates

(e.g., for benzenoids see Figure 3c, for all families see Figure S1)—when considering the O/C and H/C ratios, there are important existing differences between ASAP and DART data.

3.3 | Statistical analysis: Direct analysis against extracts

The profiling results were in good agreement with the hypothesis of a complementarity between ASAP and DART and multivariate analyses were performed for further validation. A principal component analysis illustrates a clear discrimination between DART-MS on the right side and ASAP-MS or DI-ESI-MS on the left side for PC1, whereas a certain similarity appears in negative ion mode between DI-ESI-MS and ASAP-MS in the bottom left corner, suggesting that this method might have common features (Figure S2).

For a better comparison of the compounds ionized with both sources, the most discriminant compounds between analyses are compared using Partial Least Square Discriminant Analysis (PLS-DA). The analyses, presented in Figure 4, were done with calculated exact masses and therefore allowed the comparison of all ionization techniques and of both polarities. The masses are calculated from the most representative molecular formulae corresponding to the measured masses. They had hence no error and were preserved through the binning step. These multivariate analyses further show that ASAP-MS complements DART-MS for a direct analysis of raw lichens. Indeed, in positive ion mode, DART-MS detects most of the discriminant features (Figure 4a) and is in competition with DI-ESI-MS, but ASAP-MS detects at least a specific coordinate (e.g., $m = 370$ Da, $C_{18}H_{10}O_9$). DI-ESI-MS on extract material can also miss some molecules (e.g., $m = 429$ Da, $C_{24}H_{31}NO_6$). In negative ion mode (Figure 4b), ASAP-MS and DART-MS detect mainly the same features, but DART-MS seems more efficient to reveal specific coordinates (e.g., $m = 348$ Da, $C_{15}H_{24}O_9$ or $m = 152$ Da, $C_5H_{12}O_5$). By comparison of ASAP-MS and DART-MS in both ion modes (Figure 4c), DART-MS is more efficient to detect discriminant coordinates. But ASAP-MS helps to complement with some extra detection (e.g., $m = 354$ Da, $C_{18}H_{10}O_8$).

To fulfill this exploration of coordinates without complete dereplication, propositions of identification (mainly internal metabolites depsides and depsidones) and detection of unknown compound are shown (Figures S3–S14).

From the source and detection point of view, the higher detection rate of discriminants by DART is surprising, as the primary means of ionization of ASAP and DART are quite similar.²⁴ The data are acquired on two different spectrometers with respectively time of flight and orbitrap as last MS analyzer. A comparison study between both spectrometers with ASAP source mounted could be of interest. These data also show that electrospray cannot be neglected because it highly detects discriminants—it is, after all, the golden standard with or without chromatography—so in the future it could be interesting to consider other raw material analysis methods based on ESI, such as desorption ESI.²⁵

The raw material analyses by ambient MS will not allow the detection of all the compounds found after extraction, but this process still appears to allow a good overview of the general composition of the lichen material. However, it is also worth noting that the extraction process sometimes induces chemical reactions and therefore artifact compounds.⁴

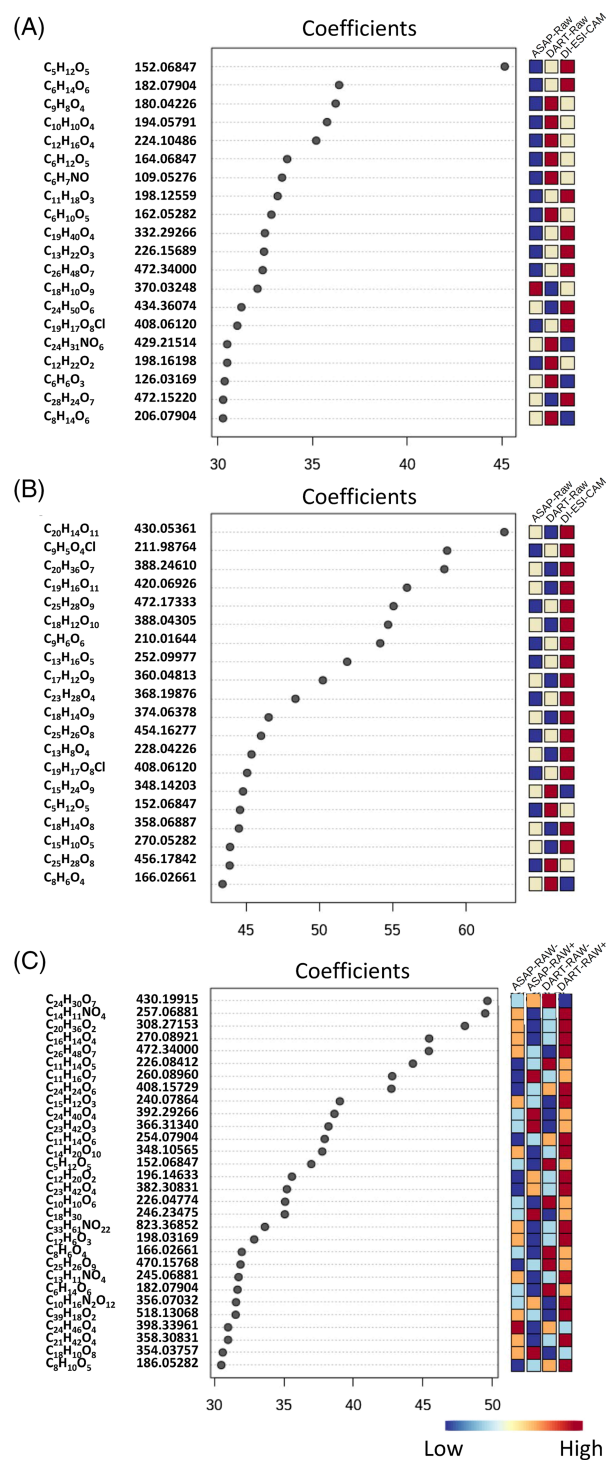


FIGURE 4 Partial Least Square Discriminant Analysis comparing: (1) the cyclohexane-acetone-methanol extracts and the raw lichen material ((A) in positive and (B) in negative ion modes, blue to red color scale) and (2) ASAP-MS and DART-MS in both polarities on the raw lichen ((C), blue to red color scale). The presented analyses were done with exact masses of chemical formulae for *Parmelia saxatilis*

This raises the question of whether both polarities should be studied in either ASAP-MS or DART-MS. Figure 4c presents a PLS-DA comparing both ionization methods in both polarities.

It shows that some compounds were only detected in ASAP-MS (e.g., $m = 354.03757$ Da) while some others were detected in DART-MS (e.g., $m = 408.15729$ Da) in both polarities.

Conversely, some compounds were detected with both methods, but were dependent on the polarity. For example, the compounds of 260, 392, 366, and 246 Da were only detected in positive mode while the compounds of 398 Da were detected in negative mode. Interestingly, two compounds (257, 308, 270, and 472 Da) were only detected in negative ASAP-MS and positive DART-MS and therefore seemed to be independent of the source and the polarity.

3.4 | ASAP or DART?

In this work, the main conclusions are that: (1) the ionization profiles returned by ASAP and DART are coherent with what can be observed on extracts, and (2) these profiles show major differences. There might be a number of reasons for these differences: it might be directly linked to the ionization—the specificity of each is likely to influence the ionization of the compounds (corona effect for ASAP, Penning ionization and ambient air plasma ionization for DART). However, one must keep in mind that ambient MS analysis techniques are *desorption-ionization* techniques and that the differences observed could be linked to the desorption component rather than the ionization component. Unfortunately, the introduction of intact lichen thalli in the APCI ion source for ASAP analysis could not be performed. The use of ground lichen is an evident bias, and in the future, it would be interesting to try to adapt our instrument to perform ASAP-MS analyses on intact samples.

Overall, ASAP-MS and DART-MS appear to be versatile and complementary methods for a direct screening of the metabolomic composition of lichens. They should be used in both polarities in order to encompass the greatest number of key features. Compared to the findings of Castilla et al.,²⁰ these results demonstrated that other ambient ionization methods can complement ASAP, and this might prove useful for metabolomics. This protocol paves the way for a large profiling method of metabolites, much easier to carry out than methods requiring an extraction step prior to the analysis, and it will need to be developed on a larger set of species and extended to other organisms.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. Further data are available from the corresponding author upon reasonable request.

ORCID

Simon Ollivier  <https://orcid.org/0000-0002-7671-1736>

Damien Olivier-Jimenez  <https://orcid.org/0000-0002-6363-0857>

Joël Boustie  <https://orcid.org/0000-0002-3936-3859>

Françoise Lohézic-Le Dévéhat  <https://orcid.org/0000-0003-4307-4942>

Nicolas Le Yondre  <https://orcid.org/0000-0002-0783-3668>

REFERENCES

1. Elix JA, Stocker-Wörgötter E. Biochemistry and secondary metabolites. *Lichen Biology*. 1996;1:154-180.
2. Stocker-Wörgötter E. Biochemical diversity and ecology of lichen-forming fungi: Lichen substances, chemosyndromic variation and origin of polyketide-type metabolites (biosynthetic pathways). In: *Recent Advances in Lichenology*. New Delhi: Springer; 2015:161-179. doi:10.1007/978-81-322-2235-4_9.
3. Choi YH, Verpoorte R. Metabolomics: What You See is What You Extract. *Phytochem Anal*. 2014;25(4):289-290. doi:10.1002/pca.2513
4. Capon JR. Extracting value: mechanistic insights into the formation of natural product artifacts – case studies in marine natural products. *Nat Prod Rep*. 2020;37(1):55-79. doi:10.1039/C9NP00013E
5. Le Pogam P, Pillot A, Lohezic-Le Devehat F, et al. Mass spectrometry as a versatile ancillary technique for the rapid in situ identification of lichen metabolites directly from TLC plates. *Lichenologist*. 2017; 49(05):507-520. doi:10.1017/S0024282917000433
6. Bertrand RL, Abdel-Hameed M, Sorensen JL. Lichen Biosynthetic Gene Clusters. Part I. Genome Sequencing Reveals a Rich Biosynthetic Potential. *J Nat Prod*. 2018;81(4):723-731. doi:10.1021/acs.jnatprod.7b00769
7. Olivier-Jimenez D, Chollet-Krugler M, Rondeau D, et al. A database of high-resolution MS/MS spectra for lichen metabolites. *Sci Data*. 2019;6(1):1-11. doi:10.1038/s41597-019-0305-1
8. Pluskal T, Castillo S, Villar-Briones A, Orešič M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics*. 2010; 11:395. doi:10.1186/1471-2105-11-395
9. Wang M, Carver JJ, Phelan VV, et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat Biotechnol*. 2016;34:828-837. doi:10.1038/nbt.3597
10. Ernst M, Kang KB, Caraballo-Rodríguez AM, et al. MolNetEnhancer: Enhanced Molecular Networks by Integrating Metabolome Mining and Annotation Tools. *Metabolites*. 2019;9(7):144. doi:10.3390/metabo9070144
11. van der Hooft JJJ, Wandy J, Barrett MP, Burgess KEV, Rogers S. Topic modeling for untargeted substructure exploration in metabolomics. *PNAS*. 2016;113(48):13738-13743. doi:10.1073/pnas.1608041113
12. Allard P-M, Péresse T, Bisson J, et al. Integration of Molecular Networking and In-Silico MS/MS Fragmentation for Natural Products Dereplication. *Anal Chem*. 2016;88(6):3317-3323. doi:10.1021/acs.analchem.5b04804
13. Gross JH. Direct analysis in real time—a critical review on DART-MS. *Anal Bioanal Chem*. 2014;406(1):63-80. doi:10.1007/s00216-013-7316-0
14. Li Z, Wang Y, Cheng Y. Mass Spectrometry-Sensitive Probes Coupled with Direct Analysis in Real Time for Simultaneous Sensing of Chemical and Biological Properties of Botanical Drugs. *Anal Chem*. 2019; 91(14):9001-9009. doi:10.1021/acs.analchem.9b01251
15. Huang Y, Huang Z, Watanabe C, Wang L. Combined direct analysis in real-time mass spectrometry (DART-MS) with analytical pyrolysis for characterization of Chinese crude propolis. *JAAP*. 2019;137:227-236. doi:10.1016/j.jaap.2018.11.030
16. Le Pogam P, Legouin B, Le Lamer A-C, Boustie J, Rondeau D. Analysis of the cyanolichen *Lichina pygmaea* metabolites using in situ DART-MS: from detection to thermochemistry of mycosporine serinol. *J Mass Spectrom*. 2015;50(3):454-462. doi:10.1002/jms.3549
17. Le Pogam P, Le Lamer A-C, Legouin B, Boustie J, Rondeau D. In situ DART-MS as a Versatile and Rapid Dereplication Tool in Lichenology: Chemical Fingerprinting of *Ophioparma ventosa*. *Phytochem Anal*. 2016;27(6):354-363. doi:10.1002/pca.2635
18. Van Krevelen DW. Graphical-statistical method for the study of structure and reaction processes of coal. *Fuel*. 1950;29:269-284.

19. Rathahao-Paris E, Alves S, Junot C, Tabet JC. High resolution mass spectrometry for structural identification of metabolites in metabolomics. *Metabolomics*. 2016;12(1):1-15. doi:[10.1007/s11306-015-0882-8](https://doi.org/10.1007/s11306-015-0882-8)
20. Castilla C, Rüger CP, Marcotte S, Lavanant H, Afonso C. Direct Inlet Probe Atmospheric Pressure Photo and Chemical Ionization Coupled to Ultrahigh Resolution Mass Spectrometry for the Description of Lignocellulosic Biomass. *J Am Soc Mass Spectrom*. 2020;31(4):822-831. doi:[10.1021/jasms.9b00091](https://doi.org/10.1021/jasms.9b00091)
21. Olivier-Jimenez D. Étude de la diversité chimique des lichens par LC-MSⁿ: acquisition et optimisation du traitement des données métabolomiques. 2021;l:47-69 (Doctoral dissertation, Université Rennes 1).
22. Hellou S, Uriac P, Le Dévehat F, et al. A chemotaxonomic study of the Xanthoparmelia pulla group in Algeria. *Herz*. 2019;32(2):485-502. doi:[10.13158/heia.32.2.2019.485](https://doi.org/10.13158/heia.32.2.2019.485)
23. Pang Z, Chong J, Zhou G, et al. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res*. 2021; 49(W1):W388-W396. doi:[10.1093/nar/gkab382](https://doi.org/10.1093/nar/gkab382)
24. McEwen CN, Larsen BS. Ionization mechanisms related to negative Ion APPI, APCI, and DART. *J Am Soc Mass Spectrom*. 2009;20(8): 1518-1521. doi:[10.1016/j.jasms.2009.04.010](https://doi.org/10.1016/j.jasms.2009.04.010)
25. Takáts Z, Wiseman JM, Gologan B, Cooks RG. Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science*. 2004;306(5695):471-473. doi:[10.1126/science.1104404](https://doi.org/10.1126/science.1104404)

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ollivier S, Jéhan P, Lambert F, et al. ASAP-MS and DART-MS as ancillary tools for direct analysis of the lichen metabolome. *Phytochemical Analysis*. 2022;33(7): 1028-1035. doi:[10.1002/pca.3156](https://doi.org/10.1002/pca.3156)