Fast Adulteration Testing of Expensive Food Ingredients with DART-HRMS: Example for Truffles

Abstract

Black truffles, due to wide price differences between the different species, are prone to be adulterated. This AppNote presents a comprehensive workflow for rapid food authenticity screening combining short acquisition times through Direct Analysis in Real-Time (DART) ionization coupled to High Resolution Mass Spectrometry (HRMS) and seamless data post-processing with the software package MetaboScape®.

Keywords:
Food authenticity, Chromatography-free, DART-HRMS, MetaboScape

Introduction

Food fraud is a major issue in the food industry leading to financial losses for food processors, inflicting lasting damage in the trust of consumers and in the most severe cases even threatening public health. With the aim for an excessive financial profit, particularly expensive products such as oils, spices and truffles are prone to adulteration. Truffles are considered a luxury product with prices ranging up to 1000 – 2000 €/kg for the Périgord truffle (*Tuber melanosporum* Vittad.). Consequently, there is a concern about adulteration, which can encompass blending authentic truffles with lower-quality alternatives or employing artificial flavorings to replicate the truffle taste. Notably, the Asian truffle (*Tuber indicum* Cooke et Massee) is morphologically highly similar to the Périgord truffle but much cheaper in price. Also, while having little flavor on its own, the Asian truffle is able to take on the flavor of other truffles when stored together. Identifying adulterated truffles is a complex challenge for the food industry. To protect the customer, an efficient, simple and fast workflow for food authenticity and quality control analysis is of high interest.

Direct Analysis in Real-Time (DART) combined with Mass Spectrometry (MS) holds a great potential for this demand. In a study by Losso et al., DART-Single Quadrupole (SQ-MS) was compared to Hydrophilic Interaction Liquid Chromatography (HILIC)-MS for the authenticity analysis of black truffles. Both techniques produced high classification and prediction scores. However, in direct comparison, DART-SQ-MS was found superior to HILIC-MS in terms of faster analysis times, lower solvent consumption and higher robustness [1]. The information depth of the resulting data can be further enhanced by using High Resolution Mass Spectrometry (HRMS) instead of SQ-MS. Aside of just classification, it enables the identification of possible markers down to a detailed elucidation of the underlying molecular structures. In the study presented here, a discrimination between the different black truffle species was achieved by unsupervised and supervised statistical models. Distinctive marker compounds were identified and annotated based on their accurate mass, isotope pattern and fragmentation pattern.

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Experimental

Truffle samples

Three different black truffle species, *T. melanosporum* Vittad., *T. aestivum* Vittad. and *T. indicum* Cooke et Massee with ten samples from each species were analyzed.

Sample preparation

Truffles were cut into pieces, lyophilized for 24 h and grounded using a mortar. 100 mg of the finely grounded truffles were weight and extracted with 1 mL of ACN:H_2O; 75:25 + 0.1 % FA; v:v:v) for 15 min at 25 °C in an ultrasonic bath. The supernatant was collected after centrifugation at 17,000 rcf for 2 min and filtered using a H-PTFE syringe filter [1]. 3 µL aliquots of sample extracts were applied onto QuickStrip® wire cards and placed into the QuickStrip sample module. Samples were spotted and analyzed as technical duplicates.

Instrumentation and software

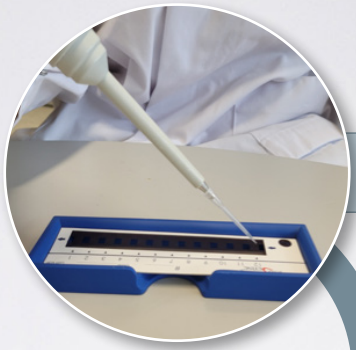
A DART JumpShot® source coupled to an impact II VIP QTOF mass spectrometer (both Bruker Daltonics) was used. Method parameters are summarized in Table 1.

Automated sample batch acquisition was carried out using Compass® HyStar 6.2 (Bruker Daltonics) with the DART source and the QTOF-MS instrument. Data evaluation was conducted with MetaboScape® 2023b (Bruker Daltonics). An overview of the experimental workflow is given in Figure 1.

Table 1.
Method parameters.

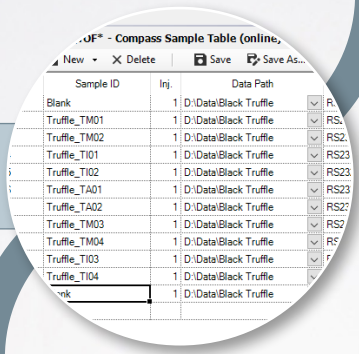
DART JumpShot Ion Source	
Sample carrier	QuickStrip wire mesh grid
Sample volume	3 µL
Ionization gas	Helium
Gas flow	Pulsed (3 s)
Temperature	300 °C
Polarity	Negative
impact II VIP QTOF Mass Spectrometer	
Mass range	*m/z* 20 - 1300
Polarity	Negative
Scan modes	Classification: MS full scan Identification: Auto MS/MS with scheduled precursor list

Experimental workflow



Pipette 3 µL of the sample extract onto the QuickStrip

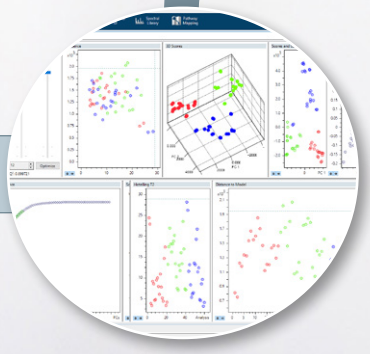
Create sample table in Compass HyStar for automated sample acquisition

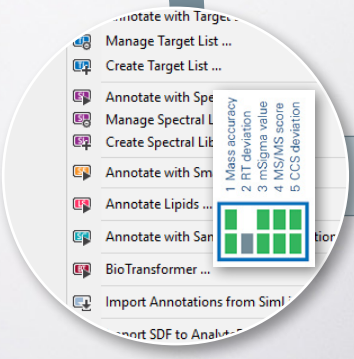


Set DART method and start data acquisition

Use MetaboScape for data evaluation

Explore the data using statistical analysis





Annotate unknowns using various annotation tools

Figure 1.

Workflow for the analysis of black truffles. It covers the data acquisition using the DART JumpShot source coupled to the impact II VIP QTOF-MS and the data evaluation in MetaboScape.

Results and discussion

Species-specific mass spectra

Figure 2 displays exemplary mass spectra for each species. Features are mostly observed in the mass range of *m/z* 80-500. Simple visual inspection of the mass spectra already reveals differences in the spectral fingerprint.

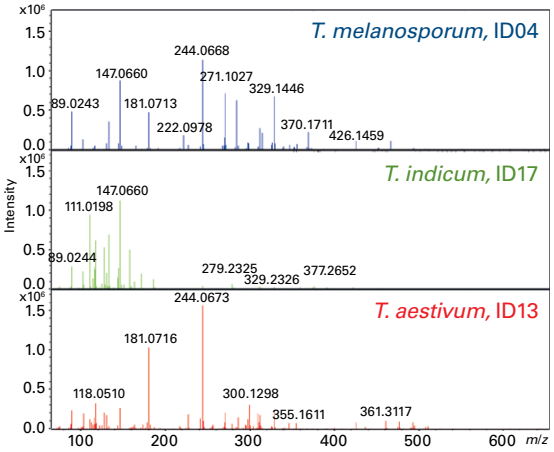


Figure 2.
Exemplary mass spectra obtained by DART-QTOF-MS in negative ionization mode.

Processing of DART-QTOF-MS data in MetaboScape

MetaboScape is a unique "all-in-one" software package for non-targeted workflows and automated identification of unknown compounds. It performs all key tasks of an untargeted screening experiment without having to export and import data from one application to the other. Using the pulsed gas flow mode of the DART source, short signals with a length of approximately 6 s were acquired. The signal shape resembles that of a flow injection peak and can readily be processed in MetaboScape for feature extraction.
Figure 3 illustrates the extracted ion chromatograms depicting the presence of the feature *m/z* 147.0661 across all samples.

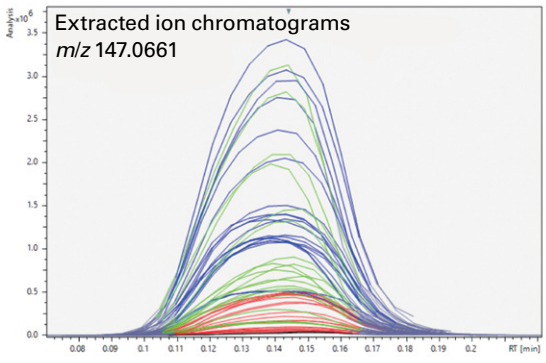


Figure 3.
Extracted ion chromatograms for *m/z* 147.0661 present in all samples.

In negative mode mainly $[M-H]^-$ ions were observed. However, adduct ions with oxygen, nitrite and nitrate which were present in the atmospheric background as well as dimers were detected in addition. This adduct formation increases the complexity of the data and may hamper the identification process. The feature extraction in MetaboScape recognizes adduct formation and respective adduct ions are automatically combined into one feature, reducing the complexity of the data significantly. This is shown in Figure 4 for the feature *m/z* 181.0715. The observed adducts are also in good agreement with those reported by Sisco and Forbes [2].

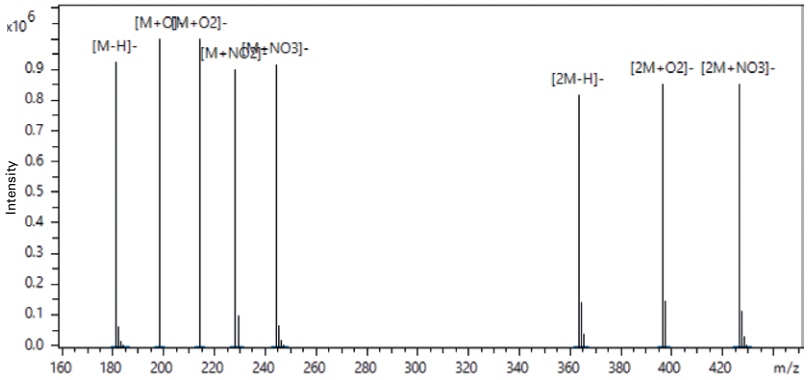


Figure 4.
Adduct ion identification and combination into one feature is automatically performed by MetaboScape. Shown here is the example for the feature *m/z* 181.0715.

Exploring the species separation with chemometrics

Various statistical tools are integrated into MetaboScape, eliminating the effort for data export and use of external software for statistical data evaluation. For pattern recognition and sample similarity analysis, an unsupervised principal component analysis (PCA) was conducted. Probabilistic quotient normalization and Pareto scaling of the data were applied in MetaboScape before building the model. Features which were present in blank samples were excluded, resulting in a total number of 332 features utilized for the statistical analysis. The results are presented in 3D and 2D score plots and the loading plot in Figure 5. Twelve principal components (PCs) were needed to explain 90% of variance of which 69% was explained by just the first four PCs. Samples from the same *Tuber* species are clearly clustered together in the 3D score plot and the possibility to distinguish the different truffle species based on the acquired DART-QTOF-MS data was successfully proven.

Using the loadings plot, features with a high influence on the respective principal component can be easily observed. For example, *m/z* 181.0715 and *m/z* 227.0679 have an impact in separating the *T. aestivum* samples from the rest. To further explore species-specific candidate markers, three Partial least squares-discriminant analysis (PLS-DA) two-class models were built, each one referring to the discrimination of a species to the other samples. Subsequently, MS/MS experiments were then performed with a scheduled precursor list containing the marker candidates from the PCA and PLS-DA analyses. From each of the PLS-DA models, the top five features according to the variable influence on projection (VIP) scoring were selected for the scheduled precursor list.

Identification of candidate marker compounds

MetaboScape includes various tools for targeted, suspect and unknown annotation. In this study, a fully automated spectral library search as well as a complete unknown annotation workflow were applied (Figure 6, A). The latter consists of a semi-automated workflow including the elemental composition prediction tool SmartFormula, structure proposals by CompoundCrawler and *in silico* fragmentation by MetFrag [3]. This 3-step workflow for the annotation of complete unknowns is illustrated on the example of *m/z* 147.0661 (Figure 6, B).

First, the elemental composition $C_6H_{12}O_4$ was proposed by SmartFormula with high confidence due to the mass accuracy of -0.46 ppm and the mSigma isotope match of 6.35. This elemental composition was then searched by CompoundCrawler in publicly available databases such as PubChem, ChEBI and ChemSpider for structural candidates. In the last step, these structural candidates were subjected to *in silico* fragmentation by MetFrag and compared with the experimental MS/MS data. Mevalonic acid was revealed as the best match with an intensity coverage of 89.6%. This acid serves as the metabolic precursor for isoprenoids which contribute to the distinctive flavor profile of truffles [4].

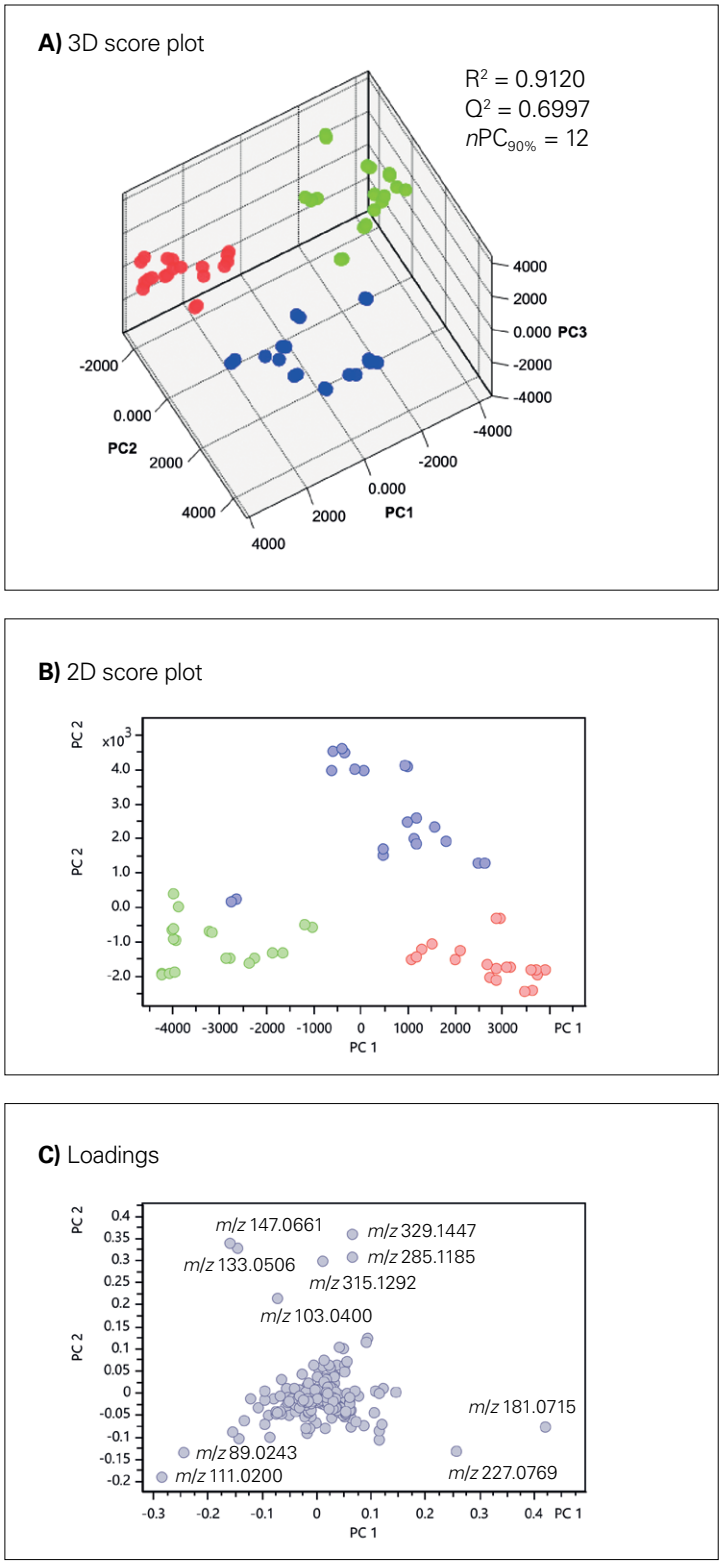
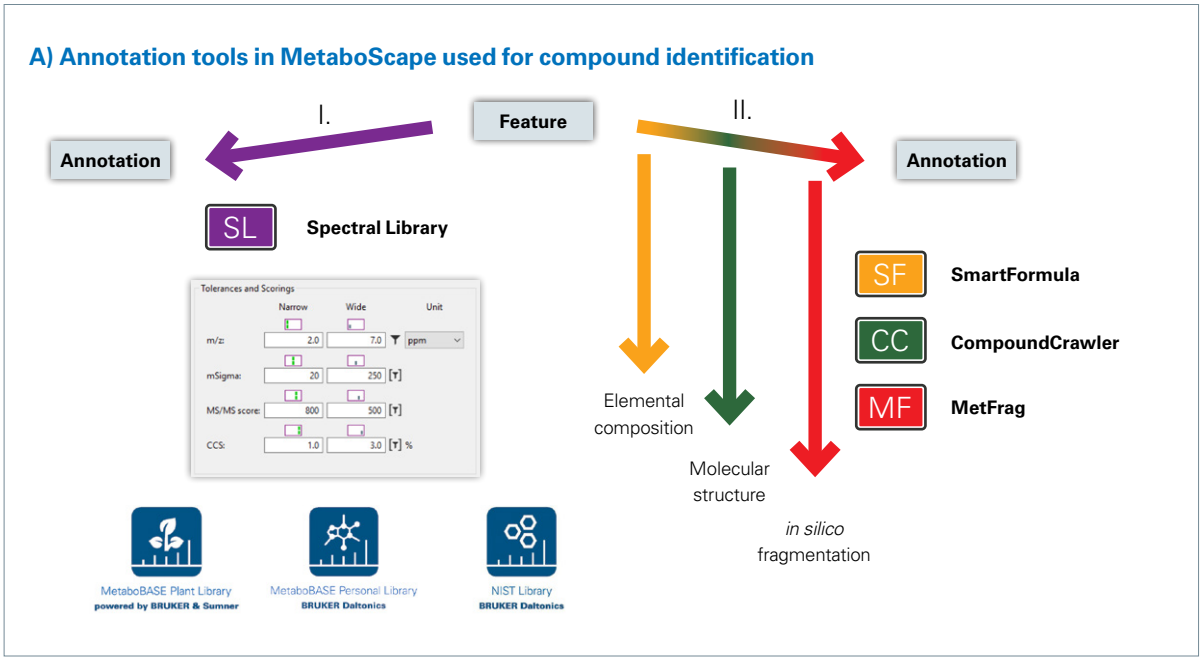


Figure 5.
Results of the Principal Component Analysis (PCA) shown as A) 3D score plot, B) 2D score plot, and C) loadings plot. Samples from the same species are color-coded: *T. melanosporum* (blue), *T. aestivum* (red) and *T. indicum* (green). Goodness of fit (R^2) and goodness of prediction (Q^2).

Using these annotation tools, six of the nine candidate markers could be assigned tentatively to a compound. Table 2 summarizes these candidate markers with their respective VIP scores as well as tentative annotation results. The plausibility of the annotations was confirmed by literature search. For the remaining unidentified candidate markers, a molecular formula could be predicted based on the accurate mass and isotopic pattern. While the fragmentation pattern highly supports the presence of polyols for final confirmation, further analysis needs to be conducted. Other compounds identified in the truffle samples but less important for species discrimination belong to the groups of amino acids, sugars and organic acids.



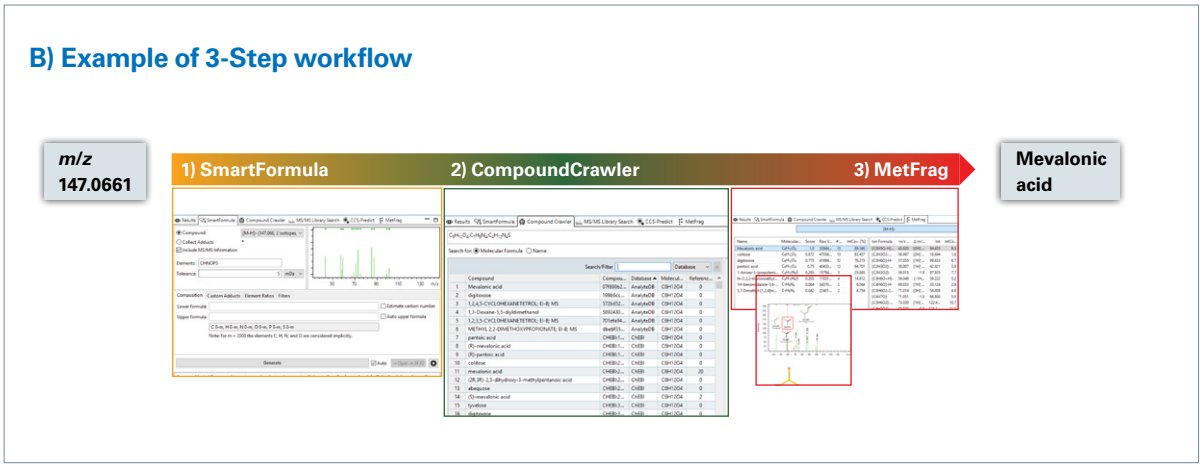


Figure 6.

Annotation tools in MetaboScape for the tentative annotation of the potential marker compounds. A) Annotation can be performed by either spectral library search (I) or a workflow for complete unknowns based on HRMS information for the accurate mass, the isotope pattern as well as MS/MS fragments (II) B) Illustration of the 3-Step workflow (II) for the feature *m/z* 147.0661.

Table 2.
Candidate marker compounds as determined by PCA and PLS-DA with putative annotation results.

PLS-DA	Feature	VIP%	Ion	Molecular Formula	Name	|ppm|	mSigma
TM vs. TI+TA	*m/z* 329.1447	6.5	$[M-H]^-$	$C_{12}H_{26}O_{10}$	Unknown 2	1.88	6.5
	m/z 285.1185	6.2	$[M-H]^-$	$C_{10}H_{20}O_9$	Unknown 1	2.30	34
	m/z 147.0661	6.2	$[M-H]^-$	$C_6H_{12}O_4$	Mevalonic acid[4]	0.46	6.4
	m/z 133.0506	5.4	$[M-H_2O-H]^-$	$C_5H_{12}O_5$	Xylitol[a, 5, 7]	0.48	9.5
	m/z 111.0200	5.0	$[M-H]^-$	$C_4H_4N_2O_2$	Uracil	0.15	4.2
TA vs. TI+TM	*m/z* 181.0715	10.1	$[M-H]^-$	$C_6H_{14}O_6$	Mannitol[a, 5-7]	0.15	0.50
	m/z 147.0661	5.9	$[M-H]^-$	$C_6H_{12}O_4$	Mevalonic acid[4]	0.46	6.4
	m/z 133.0506	5.3	$[M-H_2O-H]^-$	$C_5H_{12}O_5$	Xylitol[a, 5, 7]	0.48	9.5
	m/z 227.0769	5.2	$[M-H]^-$	$C_7H_{16}O_8$	Unknown 3	1.50	0.73
	m/z 285.1185	3.9	$[M-H]^-$	$C_{10}H_{20}O_9$	Unknown 1	2.30	34
TI vs. TM+TA	*m/z* 181.0715	10.1	$[M-H]^-$	$C_6H_{14}O_6$	Mannitol[a, 5-7]	0.15	0.50
	m/z 111.0200	7.2	$[M-H]^-$	$C_4H_4N_2O_2$	Uracil	0.15	4.2
	m/z 89.0243	6.3	$[M-H]^-$	$C_3H_6O_3$	Lactic acid[7]	1.01	0.36
	m/z 103.0400	4.8	$[M-H]^-$	$C_4H_8O_3$	(±)-3-Hydroxybutyric acid[7]	0.22	-
	m/z 227.0769	3.8	$[M-H]^-$	$C_7H_{16}O_8$	Unknown 3	1.50	0.73

[a]and/or epimers

Conclusion

The combination of DART with QTOF-MS and the software package MetaboScape is a comprehensive solution for food quality control as shown in this example for black truffles. Using the pulsed ionization mode offered by the DART JumpShot source, analysis of one sample is finished in 15 s, enabling new capabilities for high throughput analysis unmet by any chromatographic technique. Furthermore, the chromatography-free workflow highly reduces solvent consumption and limits exposure of operators to toxic solvents.

By employing Bruker's QTOF-MS instruments, candidate markers for discrimination cannot only be detected but their identity can be further elucidated based on the instrument's high mass accuracy and resolution, isotope fidelity and highly efficient and accurate MS/MS fragmentation. The time, manual effort and range of different software solutions to perform statistical analysis and annotate unknowns is significantly reduced through the combination of all these features in the "all-in-one" software MetaboScape.

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Further reading: Software workflow for identification of unknown NPS.

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