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References / further reading

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Discovering the Hidden Secondary Metabolome of Myxococcus xanthus - a Study of Intriguing Diversity
D. King, G. Zurek, O. Neuwald, M. Voit, G.J. Velarde, and H. Müller

Efficient mining of myxobacterial metabolite profiles enabled by liquid chromatography-electrospray ionization-time-of-flight mass spectrometry and compound-based principal component analysis.
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Exploratory analysis of human urine by LC-ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols.
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Combined Reversed Phase HPLC Mass Spectrometry, and NMR Spectroscopy for a Fast Separation and Efficient Identification of Phospholipid cholines.
J. Willhalm, H. Piaske, and D. Ludtke

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A strategy for the determination of the elemental composition by Fourier transform ion cyclotron resonance mass spectrometry based on isotopic peak ratios.
D. Marks, Y. Tsai, K. Nakanishi, H. Watanabe, K. Sato

Automated identification of phenolics in plant-derived foods by using library search approach.
M. Gölman-Romero, G. Zurek, B. Schröder, C. Baezmann, A. Segura-Carretero, and A. Fernandez-Gutierrez

Metabolomics has emerged as an exciting new area of biological research and gained substantial attention in recent years. Previously, significant efforts have been made to probe and understand the genomes, transcriptomes and proteomes of a variety of organisms. Despite massive investments to sequence and identify cellular DNA, RNA and proteins, our understanding of many key biological functions remains rudimentary and incomplete. Many researchers believe that perhaps more complete answers can be gleaned from studying small molecules that are utilized for a variety of metabolic and cellular functions. Because of this belief, many laboratories, Metabolomics is catching up with traditional Proteomics and Transcriptional efforts and has been christened “Biochemistry’s new loss” [1].

Typically, during a Metabolomics study, a large number of cell or patient samples are analyzed and compared to identify potential biomarker compounds which correlate to a disease, drug toxicity, or genetic or environmental variation. Validated biomarkers can form the basis for new diagnostic assays, and lead the way to help establish truly personalized medicine, since changes in small molecule concentrations are closely related to the observed phenotypes.

Answering the Challenges of Metabolomics

Metabolomics researchers are regularly confronted with the daunting tasks of acquiring large sets of data and then analyzing them in minute detail. Due to the chemical diversity of small molecule metabolites, it is impossible to study the entire metabolome using a single analytical technique or technology. Comprehensive analysis of the metabolome requires multiple solutions.

The Metabolome’s chemical space can be fully explored by utilizing Bruker’s high performance LC-MS, GC-MS, CE-MS, and NMR systems in conjunction with dedicated software for data evaluation.

One of the most challenging tasks in the Metabolomics studies is the identification of unknown compounds. Exact mass and isotope pattern information in MS and MS-MS spectra acquired by the solarX (FT-MS) or micrOTOF series and mXis instruments can be used to definitively identify unknown compounds. The intrinsic capabilities of these instruments in conjunction with the SmartFormula3D software enables accurate and reliable molecular formula generation for a wide variety of unknowns.

In order to enhance separation and increase throughput, many laboratories have turned to utilization of UHPLC for Metabolomics studies. Since the mass accuracy, isotopic fidelity and resolution of mXis and micrOTOF-series ESI-Q-TOF instruments are independent of the acquisition rate, they are perfectly suited for a coupling to UHPLC separations. By utilizing a Bruker solution, their high-resolution and accurate mass LC-MS capabilities enable both targeted and untargeted Metabolomics work-flows. In addition, the broad dynamic range characteristics of these systems allows both, high- and low-abundance compounds to be studied within the same dataset – even within the same mass spectrum.

Finally, Bruker offers the capability to integrate both LC-MS and NMR techniques into a single analytical system for maximum metabolite coverage and molecular identification. The MetaboLinkProfiler is a powerful combination of techniques and is exclusively designed to address all analytical needs for metabolic profiling and structure elucidation.

This poster hall is a “snapshot” of some of the current work utilizing a variety of Bruker solutions for Metabolomics studies. We would like to thank all of our partners and customers for their constant input and feedback. This information has served as an invaluable guide for us in our solution development efforts. We would like to extend our special thanks to everyone who has been involved in the generation of the outstanding posters compiled in this poster hall for your benefit.

Sincerely yours,

Aiko Barsch
Market Manager Metabolomics


Powerful Solutions Tailored for Metabolomics Applications
The Need for Speed in Metabolomics: UHPLC with maXis MS Analysis of Tea Extracts

Untargeted metabolic profiling of different black and green tea extracts was performed using a UHPLC and maXis UHR-TOF analytical system. Processed data was submitted to principal component analysis (PCA) to identify compounds differentiating the tea varieties. The compounds’ sum formulae were queried in public databases, which enabled the identification of compounds characteristic for green and black tea.

Introduction

Fast separation of complex samples using high-resolution chromatography and state-of-the-art mass spectrometry are required to meet the simultaneous need for high sample throughput and high-quality data in metabolomics. Hypetization of UHPLC and the maXis UHR-TOF EXTRA High Resolution Time-of-Flight mass spectrometer deliver speed without compromising on any performance factor, such as sensitivity, mass accuracy or resolution.

Black and green tea account for more than 95% of tea consumed worldwide. Health benefits have been hypothesised for both types of tea. A deeper understanding of potential health promoting effects – as well as an improvement in quality and taste – is a topic of interest in academia and the food industry.

Data generated from different tea extracts via UHR-TOF MS was subjected to statistical analysis. Highly accurate mass data and isotopic pattern information in MS and MS-MS spectra enable reliable sum formula generation. Combining these formulas with database queries facilitates identification of unknown compounds – often described as the major bottleneck in metabolomics.

Experimental

In brief, 14 different tea extracts (black, green and 2 Darjeeling) were prepared by steeping tea samples in 100 mL hot water for 5 min. Analysis was performed with an Ultimate 3000 Rapid Separation System (Bruker) and maXis UHR-TOF (Bruker Daltonik) operating in ESI negative mode liquid acquisition rate 4 Hz. Data pre-processing and feature extraction were performed with Compass DataAnalysis; Principal Component Analysis (PCA) and sum formula generation were performed using SmartFormula in ProfileAnalysis (Bruker Daltonics).

Complete experimental conditions are given in Reference [1].

Results and Discussion

LC-MS data obtained with the different tea extracts were processed using the “First Molecular Feature” (FMF) peak detection algorithm, which efficiently differentiates real signal from background noise. FMF compounds were used as input for PCA analysis.

The PCA score plot (Figure 1) shows a clear separation of the data points according to their oxidation level. The PCA score plot (Figure 1) shows a clear separation of the data points according to their oxidation level. The PCA score plot (Figure 1) shows a clear separation of the data points according to their oxidation level. The PCA score plot (Figure 1) shows a clear separation of the data points according to their oxidation level.

Several PAF compounds contributing to the differences between the tea types are highlighted in the loadings plot (Figure 1). Sum formulae were calculated using Smartformula, which provides a ranking according to the best fit of measured and theoretical isotopic pattern within a specified mass accuracy window. The quality of the isotopic pattern fit is expressed in the mSigma value (Table 1) provided in the SmartFormula output. All elemental compositions given in Table 1 represent the first sum formula within 1 ppm mass accuracy window. A query of the sum formulae in public databases using the CompoundCrawler enabled a tentative identification of these compounds.

Table 1: Selected loadings differentiating the tea samples.

<table>
<thead>
<tr>
<th>Loading</th>
<th>Mass (ppm)</th>
<th>Abundance</th>
<th>Identity of the compound</th>
<th>MS-MS spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>191.0560</td>
<td>6.7 C_{6}H_{8}O_{4}</td>
<td>Trigalloyl-glucose</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>307.0826</td>
<td>10.6 C_{6}H_{8}O_{4}</td>
<td>Gallocatechin</td>
</tr>
<tr>
<td>3</td>
<td>7.4</td>
<td>289.0718</td>
<td>-0.24 6.1 C_{6}H_{8}O_{4}</td>
<td>Epicatechin-3-gallate</td>
</tr>
<tr>
<td>4</td>
<td>2.8</td>
<td>483.0780</td>
<td>-0.39 6.8 C_{6}H_{8}O_{4}</td>
<td>Theogallin</td>
</tr>
<tr>
<td>5</td>
<td>6.9</td>
<td>433.0721</td>
<td>0.35 13.7 C_{6}H_{8}O_{4}</td>
<td>Caffeic acid</td>
</tr>
<tr>
<td>6</td>
<td>6.7</td>
<td>441.0648</td>
<td>0.22 8.4 C_{6}H_{8}O_{4}</td>
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</tr>
</tbody>
</table>

Assignments based on m/z MS–MS data can be made with a high level of confidence as demonstrated by the sub-mDa mass deviations shown in the Figure 2. The structure of the candidate is consistent with the observed cleavages of ester and neighboring bonds of the gallic acid-sugar conjugate Trigalloyl-glucose.

Conclusion

Fast UHPLC separation coupled to maXis UHR-TOF-MS analysis enabled the comprehensive analysis of complex mixtures of small molecules, namely black and green tea samples. PCA analysis enabled differentiation of tea samples according to their oxidation level.

The sub-pm mass accuracy and the True Isotopic Pattern information delivered by the maXis UHR-TOF-MS instrument facilitated the straightforward sum formula generation of compounds characteristic for different types of tea. By combining accurate mass and isotopic pattern information in MS and MS–MS spectra, SmartFormula2D facilitates the faster interpretation of fragmentation data. Additionally, SmartFormula2D extends the capability for unique sum formula generation to compounds with higher molecular weight.

Used in combination with CompoundCrawler database queries, the feasibility of the UHPLC and maXis UHR-TOF analytical approach for the identification of unknown compounds in metabolomics studies has been demonstrated.

References


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Plant Metabolomics

Development of an HPLC-TOF-MS screening-platform to assess ppHypSys-dependent metabolic changes in Nicotiana attenuata during insect herbivory

Matthias Schüttler1, Eva Rotha1, Emilsson-Gaquerol2, Beatrice Berger1, Alice Barthe1, Birgit Schneider3, Robert Fazelter4, Gabriela Zurek5, Ian T. Baldwin6
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2 Bruker Daltonik GmbH, 23159 Bremen – Germany
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Introduction

A reliable HPLC-TOF-MS-based metabolomics platform to screen for chemical changes in leaf extracts of Nicotiana attenuata using simulated insect attacks W+OS (W = mechanical wounding, OS = application of Manduca sexta oral secretions) is presented. The platform was used to investigate the role of the hydroxynorvaline-rich glycosylated systems precursor ppHypSys in a signaling molecule during insect herbivory. After thorough internalization of the FPA signals and transcript levels by RNAi (Silkies) or over-expressing the ppHypSys precursor under the control of a 35S-promoter (35S), we compared metabolic changes of wild-type (WT), Silkies and 35S in response to W+OS.

Methods

ESI-Qq-TOF-MS was applied to M. sexta extracts, and peak finding was performed using the FindPeaksCompounds peak find delivering isotopic as well as chromatographic information.

Results

The identification of regulated metabolites can be achieved by comparing the ion scores and/or loadings on PC1 (A). Kinetic plots for this ion showed that plants silenced for ppHypSys accumulated this compound (B) during herbivory. The peak of interest was then localized in the original data (C) and the elemental composition was calculated using both the accuracy mass and isotopic pattern information (D).

Conclusions

- The proposed method combining an ESI-Qq-TOF-MS based metabolomic screening with statistical analysis, kinetic profiles and subsequent identification was successfully applied to a large plant metabolomics data set investigating the influence of ppHypSys during insect herbivory.

- The nitrogen containing molecules are currently independently explored using WT plants grown in N-free media. In a next step experiments using accurate and high resolution HILIC/MS data and classical NMR analysis will be carried out.

Quality Control

The reproducibility of the method was checked by analysis of the QC samples and analysis of replicates. The reproducibility was assessed by comparing the average response of the ion spectrum of the compound C13H19N2O3 [M+H]+ selected in the original data (C) and the simulated spectrum with annotated formulae. The precision of the method was monitored.

- By means of internal standards and a pooled quality control the next step experiments using accurate and high resolution HILIC/MS data and classical NMR analysis will be carried out.
We demonstrate that differences in the intraspecies variation of secondary metabolism production by Myxococcus xanthus using MS analysis of wildtype and mutant strains is a promising method to uncover novel compounds. Analysis of crude extracts from Myxococcus xanthus revealed considerable deviation within replicate fermentations, which accounts in part for the low degree of shared metabolites. To overcome this obstacle, we employed probabilistic features and accurate mass measurements for the extraction of metabolite profiles. Tenfold replicated LC-MS analysis was integrated in the range (100-2000 m/z) by using a BPC to comprehensively evaluate the metabolite profiles and identify novel secondary metabolites. A probabilistic bucketing approach for PCA of metabolite profiles was established to find similarities and differences among the strains, which can help to uncover the assumed hidden metabolites.

**Results**

LC-MS data was prepared for PCA analysis in ProFinder (Bruker) using a bucketing approach (Fig. 2). The LC-MS data was integrated from 0.2 to 2000 m/z and bucketed in 0.01 Da (m/z) and 0.05 Da (m/z). Each sample was integrated as one bucket. The integrated mass data was then used for the extraction of metabolite profiles. In order to explore the variation in the strains, four wildtype strains of Myxococcus xanthus were grown in YES plates, and the metabolite profiles were extracted by using Profinder. The wildtype strains exhibited considerable deviation within replicate fermentations, which accounts in part for the low degree of shared metabolites (Fig. 2).

**Conclusions**

We demonstrate that differences in secondary metabolism production can be extracted by PCA, even using small diagnostic variations within replicate fermentations. It is shown that accurate mass and isotopic pattern data from ESI-TOF analysis can be readily applied to their metabolite profile. A probabilistic bucketing approach for PCA of metabolite profiles is a promising method to uncover novel compounds. This method is presented in a high resolution scan (2).

**Materials and Methods**

**Analytical equipment**

- LC-MS analysis was performed using Bruker’s UHR TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).
- Sample processing was performed with ESI-TOF MS data from a Bruker Daltonik platform.
- Data were acquired using the ProFinder software (Bruker Compass, 2001).
- MS data was evaluated using the Pfinder (Bruker) software package.
- ESI-TOF MS data was used for MS and shows to evaluate data and bucketing multiple ternary samples.
Challenges in Metabolomics addressed by targeted and untargeted UHR-Q-TOF analysis

Gabriela Zurek*1, Alio Barciz2, Wibabo Lahana2, Bastian Krug2, Ralf Mutters2
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2 Institute for Plant Systematics and Biochemistry, University of Bremen, 28359 Bremen, Germany

Introduction
Metabolomics is an emerging field enabling the investigation of target metabolite pools in a wide range of samples, such as tissue extracts, plasma, serum, saliva, urine, and others, and is becoming increasingly important for today’s pharmaceutical research. This field is particularly useful for understanding the biological impact of diseases, the activity of different drugs or therapeutic agents, and the effects of variations in the lifestyle of the patient. However, metabolomics is a challenging field characterized by high data volume and complexity. The number of metabolites identified in a typical metabolomics study is often in the order of thousands or millions, presenting a great challenge for data analysis and interpretation.

Here, we present an approach to UHR-Q-TOF based analysis of different metabolite classes using a combination of targeted and untargeted approaches to solve these challenges. We employ the available high-resolution mass spectrometry to achieve high sensitivity, accuracy, and speed. The automated target analysis module of the Compound Discoverer software is used to analyze sample classes, and the preparation of the data for targeted and untargeted approaches is performed.

Methods
1. Sample preparation and analysis were performed using the Bruker UHR-Q-TOF mass spectrometer equipped with an ESI source. The compound identification was performed using the Compound Discoverer software.
2. Sample set: The sample set consisted of different Myxobacterium species with different genetic backgrounds and growth conditions.

Targeted Analysis
The targeted analysis is focused on known metabolites using retention time, accurate mass, and isotopic pattern information to identify and quantify the metabolites. The data is acquired using positive ionization mode (ESI+).

Untargeted analysis using PCA
The data set can be divided into different classes (e.g., positive ionization mode (ESI+)). In this study, we used a high-performance liquid chromatography (HPLC)-MS method to separate the metabolites, and the data was acquired using the Bruker UHR-Q-TOF mass spectrometer.

Sample Set
The sample set consisted of different Myxobacterium species with different genetic backgrounds and growth conditions. The samples were prepared by extraction with methanol and analyzed by HPLC-MS, with the data acquired using the Bruker UHR-Q-TOF mass spectrometer.

Results
The targeted analysis identified 10 different metabolites, while the untargeted analysis identified 20 different metabolites.

Conclusions
The targeted analysis is useful for identifying known metabolites, while the untargeted analysis provides a comprehensive overview of the metabolites present in the sample. The combination of these approaches allows for a deeper understanding of the metabolic pathways and processes involved in different Myxobacterium species.

References

Characterization of black and green tea using ESI-Q-TFQ-MS and data evaluation by principle component analysis

Aiko Barci1, Gabriela Zurek1, Wibabo Lahana1, Bastian Krug1, Ralf Mutters1
1 Institute for Plant Systematics and Biochemistry, University of Bremen, 28359 Bremen, Germany

Introduction
Black and green tea represent more than 95% of all tea consumed throughout the world. They have a wide range of health effects, including antioxidative, anti-inflammatory, and anti-cancer activities. However, the health effects of these teas are not fully understood, and the analysis of their metabolites is a complex task.

This study characterized black and green tea using ESI-Q-TFQ-MS and data evaluation by principle component analysis. The tea samples were extracted and analyzed using liquid chromatography coupled with mass spectrometry (LC-MS). The data was acquired using the Bruker UHR-Q-TOF mass spectrometer.

Results
Black tea and green tea were characterized by different metabolites. The black tea contained higher amounts of catechins compared to green tea, while the green tea contained higher amounts of flavonoids.

Conclusions
The characterization of black and green tea using ESI-Q-TFQ-MS and data evaluation by principle component analysis provides a comprehensive overview of the metabolites present in the tea samples. This study highlights the potential of metabolomics for understanding the health benefits of these teas.

References

Food & Nutrition

Characterization of black and green tea using ESI-Q-TFQ-MS and data evaluation by principle component analysis

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Introduction
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References
Analysis of Whisky by Electrospray FT-ICR Mass Spectrometry: Proof of Origin by Statistical Methods

Mathias Witt, Rainer Pace, Jann-Fusscher and Jochen Friedrich

Bruker Daltonik GmbH, Bremen, Germany

Introduction
Whisky is a high-class consumed alcoholic beverage with a several billion dollar market. Due to the high value of this market, many attempts have been made to understand the origin of different whiskies and improve the process of distillation. The proof of the origin of this highly valued beverage is of great interest to consumers, producers, and law enforcement.

Methods

1. Metabolomics Posterbook 2011

Food & Nutrition

Metabolomics

Metabolic Profiling and Fingerprinting of Beverages using LC-MS and NMR

M. Gospodinov*, E. Humpf*; H. Schwerkow*: P. Rinke*, G. Zurek

*M. Gospodinov, E. Humpf, G. Zurek, Bruker BioSpin GmbH, Rheinstetten, Germany; *G. Zurek, Institute of Food Chemistry, German Research Center for Food Safety, Bremerhaven, Germany

Introduction

LC-MS and NMR can provide a comprehensive set of data. While LC-MS/MS shows best results for medium polar, medium weight compounds at lowest concentration, the NMR is able to identify extremely polar, small and medium size molecules. Mass spectrometry can measure the molecular weight at the total volume of a regular GC chromatography. Therefore, the results in suppression of electrospray ionization caused by extraction with improved and high sensitivity.

Reducing variables for statistical analysis

For both, LC-MS and NMR all entries in this dataset table can be screened on a three dozens of features and by a PCA-DA approach to the third discriminating ncv and ypm variables for the two combined clusters of Chinese and French apple juice and with the exclusion of the IRT

Most discriminating variables for LC-MS

The most discriminating ncv (intensity) - retention time - exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm. The most discriminating variables for LC-MS/MS are shown for the most discriminating ncv with exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm. The most discriminating variables for LC-MS/MS are shown for the most discriminating ncv with exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm. The most discriminating variables for LC-MS/MS are shown for the most discriminating ncv with exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm. The most discriminating variables for LC-MS/MS are shown for the most discriminating ncv with exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm. The most discriminating variables for LC-MS/MS are shown for the most discriminating ncv with exclusion range 0.05 ppm; 8.75 ppm; exclusion range 0.1 ppm.

Results

LC-MS and NMR provide a comprehensive set of data. While LC-MS/MS shows best results for medium polar, medium weight compounds at lowest concentration, the NMR is able to identify extremely polar, small and medium size molecules. Mass spectrometry can measure the molecular weight at the total volume of a regular GC chromatography. Therefore, the results in suppression of electrospray ionization caused by extraction with improved and high sensitivity.

Conclusions

The combined acquisition of LC-MS and NMR data for a large set of similar samples together with a multivariate statistical analysis allows the easy differentiation of juice from different origins. Moreover, it can also accurately identify the different regions and the highest concentrations in the juice sample.

Summary

Jasmin from different geographical origins were analysed by a combined LC-MS and NMR approach. The statistical analysis directly identifies the most important discriminating features for each origin. Furthermore, a combined statistical approach reveals inferences of Jasmin as being one of the main discriminators.
RNA, particularly t-RNA, contains a number of modified nucleosides placed post-transcriptionally within the polynucleotide molecule by various modifying enzyme systems such as methyltransferases and ligases. During RNA turnover, free normal and modified nucleosides are formed by hydrolytic action of ribonucleases and phosphate-elimination by phosphatases.

A mixture of 20 known nucleosides (concentration from 0.8 to 640 nmol/ml based on urine sample values) as well as purified real samples were introduced into the LC-ESI-oa-TOF-MS measurements. In case of an aromatic base in the nucleoside, it is clearly observed that the urine samples contain more nucleosides than the standard mixture.

Potential tumor markers in cancer diagnosis.

The smaller the sigma value the better the fit. Figure 3 shows the molecular formula calculated from the mass spectrum of 1-methylguanosine. Table 1 contains a selection of identified structure proposals. The search was restricted to ribosyl-containing nucleosides including affinity chromatography purification (AffiGel601, BioRad, USA) and reversed phase chromatographic separation of the prepurified RNA-metabolites. Confidence into the presented method was evaluating both accurate mass position and true isotopic patterns for the calculation of molecular formulae.

Combined analysis of NMR and LC/MS data: observations in urine samples from a lifestyle study.

Effect of Smoking

Fig. 5: Top: PCA analysis of LC-MS data from control spectrum taken from middle of scores plot and MS & complete data evaluation.

Combined Consistency Matrix

Fig. 5: Top: PCA analysis of LC-MS data from control spectrum taken from middle of scores plot and MS & complete data evaluation.

References

[1] Kammerer, B., Frickenschmidt, A., Maldi -TOF-MS analysis of urinary metabolites. Confidence into the presented method was evaluating both accurate mass position and true isotopic patterns for the calculation of molecular formulae.

While mass spectroscopy is giving best results for polar analytes eluting with a reasonable retention from the HPLC column, it does not allow the detection of small endogenous metabolites (Fig. 3). While the MS response of an analyte usually includes the pseudomolecular ion and its isotopic distribution, the MS/MS spectrum has to be considered to determine the identity of the analyte. For this purpose, the fragmentation pattern of related compounds is used to establish a database, which already comprises more than 500000 different fragments.

While there is an increasing demand for high throughput analyses, the use of automated software tools will become crucial. The Bruker MS & complete data evaluation software AMIX 3.6a (Bruker Biospin) is particularly suited to the analysis of high throughput datasets. Although the main focus of our work is the identification of potential biomarkers of breast cancer, the presented method can be applied to other diseases as well. The MS/MS fragmentation patterns of known compounds were compared with the MS/MS fragmentation patterns of the compounds identified by MS with the aid of AMIX. While AMIX is allowing to search only ribosyl-containing nucleosides, the combination of NMR and LC/MS spectra, can in principle be identified by the cross-peaks in the three dimensional HSQC spectrum. The detection system in case of an aromatic base in the nucleoside.

When doing distribution comparison of all metabolites from 283 samples by PCA, the distribution for 328 m/z and 7.34ppm of all samples can be found which might be explained by the covariance analysis.

Fig. 2: PCA on LCMS Data, see text for details

Fig. 3: PCA on NMR Data, see text for details

Fig. 4: Scores Plot Loadings Plot

Fig. 5: Spectra taken from end of blue vector

Fig. 2C: True isotopic pattern

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Fig. 4: Scores Plot Loadings Plot

Fig. 5: Spectra taken from end of blue vector
Elemental analysis of fulvic acids of Shilajit by ultra-high resolution FTMS

Matthias Wilt1, Khalid Anwer1, Sotiris Koch2 and Jens Fuchser1

1Mineral Technologies Gmbh, Bremerhaven, Germany
2Dhirubhai Ambani Institute of Chemical Technology, Mumbai, India

Introduction

Shilajit is a dark-brown extrudate of variable consistency, oozing out from layer of rocks in many mountain ranges all over the world. It is made of organic material having been exposed to high pressures and high temperatures. Shilajit has been used as a rejuvenator and an adaptogen for thousands of years. Its major physiological action has been attributed to the presence of bioactive dibenz-alpha-pyrones along with humic and fulvic acids which act as carrier molecules for the active ingredients. Fulvic acids are known as a group of oxygen containing polyelectrolytes of organic compounds. However, due to the extremely high resolving power and mass accuracy of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry the elemental composition of these compounds can be determined directly from this complex mixture.

Methods

Mass spectrometer: APEX Qq Q-TOF (Bruker Daltonics, Bremen, Germany) using positive electrospray ionisation. Analyte: Fulvic acids of Shilajit after desalting on silica gel using a dried exchange resin and dialysing with distilled/ultrapure water/analyte adsorber: The used solution was approx. 0.5 mg/mL in water/methanol 50/50 sprayed @ 0.12 mL/h.

Results

Figure 1 displays the complexity of the sample. Automated assignment of molecular formulae was carried out using TargetAnalysis. For the analyses different number of nitrogen atoms were allowed.

Figure 2 illustrates the need for high resolution mass spectrometry: A resolution of 100,000 is insufficient to completely resolve all present compounds.

Conclusions

• From the measurement more than 5000 elemental compositions could be identified in the fulvic acid extract of Shilajit.
• For complex mixtures, which are not separated sufficiently by chromatographic methods, high resolution (e.g. 350,000) FTICR MS is mandatory.
• Similar distributions of elemental compositions have been found for compounds bearing no, one or two nitrogen atoms.
• The measured average C/H ratio of 1.27 is relatively high compared to the one of Guayacan river fulvic acids standard from the IHS (1.02).
Characterization of dissolved organic matter in marine pore waters by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Methods

52 ml of pore water was obtained from the surface sediment by piston sampling. DOM was extracted from the pore water and the river water by solid phase extraction (Dianaph, Pl). Samples were ionized by negative electrospray ionization and detected with a 5-4 T FT-ICR mass spectrometer. Unusual molecular formulas were determined on the base of exact masses. Ions with molecular formulae of form DOM/MON (2:1) by microwave and analyzed by GC-MS/MS for the identification and quantification of the compounds.

Results

We identified distinct molecular changes from the river to the pore water DOM and linked them to either source variations or transformation processes. River DOM:

- High O/C and low H/C values (Fig. 3a)
- Source signal: higher complexity and oxygenation of terrestrial DR

Pore water DOM:

- Higher abundance of R-containing molecule and compounds with lower H/C (Fig. 3b)
- Source signal
- Progressive loss of fulvic acids and first appearance of compounds with H/C ratios (Fig. 3a) along the transect
- Source signal

By weighted average m/z & DD compared to the marine and river water column (Fig. 3b)

Transformation of DOM induced by a higher microbial activity in the sediment. Within the pore water DOM the transformation step seemed to be linked to sediment properties (e.g. grain size, TOC; data not shown) and therefore potential substrate for microbes.

Conclusions

- The molecular compositions of DOM in marine pore water and of river DOM were determined by FT-ICR-MS.
- Molecular variations in DOM could either be linked to a change in source (DOC, H/C) or to transformation processes (O/C, H/C, m/z<500).
- DOM gradient within the sediment.
- While the analyzed DOM represents a recent marker, POM is more stable in marine sediment; degradation of POM becomes more important with longer transport and residence times.

Conclusions

- The study demonstrated that FT-ICR-MS is able to resolve the molecular composition of DOM, POM and its degradation along a river to marine transition. These data are necessary for understanding the organic matter cycle at continental shelves. The MetabolicProfiler™ introduced in 2010 combines a liquid chromatography, a 600 MHz NMR spectrometer with flow probe and a LC-ESI-QTOF MS all under control of an oracle based order management system SampleTrack™.

The MetabolicProfiler™ introduced in 2010 combines a liquid chromatography, a 600 MHz NMR spectrometer with flow probe and a LC-ESI-QTOF MS all under control of an oracle based order management system SampleTrack™.

- The complete process with preparation, acquisition and data processing is fully automated.
- Protocol based data processing is done in the background.
- The MetabolicProfiler™ supports the analysis of 1–100 samples per day. The database contains over 25,000 compounds and the NMR spectral database contains over 40,000 spectra.

Applications & References

- ESI-QTOF MS of modified tobacco plants, M. Schröder & J. Bahlman, MPI Chemical Biology, Germany.**
- Screening of myxobacterial extracts using UPLC & ESI-QTOF-MS, D. Krug & B. Müller, Uni Saarbrücken, Germany.**
- Personalized Metabolite Profiling using NMR, CEBRE Florence, Italy.
- Newborn screening by high resolution flow NMR, C. Panch & B. Nauck, University Hospital Greifswald, Germany.
- Fruit Juice Screen™ & GF & Bruker BioSpin for Quality Assurance, F. Rink, SFG, Germany.

** Posters on display at this meeting.

Fig. 1. MetaboliteProfiler system of flow chamber containing carbohydrate and hydrocarbon mixture with the MetabolicProfiler™ LC-ESI-QTOF MS system.

Fig. 2 The TOF MaXis (Bruker BioSpin) with the ISQ8 in <2 min acquisition time.

Fig. 3 The novel ultra high resolution (UHR) TOF MaXis™ provides the highest resolution combined with high mass measurement accuracy and signal to noise ratio. They enable the complete search and identification of compounds. This enables the scientist to identify compounds and to generate a list of putative candidates. The NMR spectral database contains over 40,000 spectra. The MetabolicProfiler™ and the database are essential for metabolomics analysis. The metabolomics data is summarised in a single comprehensive reporting system.
Metabolomics Technology: LC-MS-NMR

**Integration and Robustness: a Touchstone in Metabolomics Studies**

Markus Gudeljanov1, Silke Keller1, Bernd Hartig2, Eleonora Schindler1, Christian Frohnhöfer2, Christian Hausinger2, Hans Peter Barz3, Corinna Bassmann3, Manfred Spraul2

**Brucker BioSpin GmbH, Rheinstetten, Germany**

**Brucker Daltonics GmbH,remen, Germany**

**Introduction**

When integrating multiple analytical techniques like LC, APCI-MS, and NMR into an integrated platform, it is especially important to establish robustness and reproducibility. The platform was designed to allow the generation of high-quality spectra from multiple test samples in order to control the optimization process taken by APCI-MS and NMR. In addition, it is crucial to develop new applications for the platform in order to explore its capabilities fully. In this study, we investigated the performance of the platform using two different human urine samples and compared the results obtained by APCI-MS and NMR.

**Methods**

The platform features a liquid handler (MCI), a liquid nitrogen tank, and a liquid chromatograph (Bruker Daltonics).

**Liquid Handler**

- Ability to deliver up to 80 samples
- Liquid nitrogen tank
- Liquid chromatograph

**LC-MS-NMR Platform**

- Spectrometer (Bruker BioSpin, Bremen, Germany)
- Mass spectrometer (Bruker Daltonics, Bremen, Germany)
- Liquid nitrogen tank
- Liquid chromatograph

**Results**

- Reproducibility of the automated liquid handler was excellent, with RSD values of peak intensities in the LC-MS chromatogram being < 3% without preparation and < 5% within the same initial urine.

**Conclusion**

The automated liquid handler provides excellent results, with RSD values of peak intensities in the LC-MS chromatogram being < 3% without preparation and < 5% within the same initial urine.

**Comparison of PCA results**

<table>
<thead>
<tr>
<th>NMR</th>
<th>LC-MS</th>
<th>NMR vs. LC-MS</th>
</tr>
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<tr>
<td>A</td>
<td>B</td>
<td>A vs. B</td>
</tr>
<tr>
<td>C</td>
<td>D</td>
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</table>

**Table 1**

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>[M+H]+</th>
<th>M+</th>
<th>TMS+</th>
<th>H</th>
<th>m/Cl (mDa)</th>
<th>Error</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>183</td>
<td>1</td>
<td>3</td>
<td>26</td>
<td>225.0979</td>
<td>0.25</td>
<td>5.7</td>
</tr>
<tr>
<td>Phenyl-Gly</td>
<td>197</td>
<td>1</td>
<td>0</td>
<td>31</td>
<td>225.0979</td>
<td>0.25</td>
<td>5.7</td>
</tr>
<tr>
<td>Caffeine</td>
<td>197</td>
<td>1</td>
<td>0</td>
<td>31</td>
<td>195.1102</td>
<td>1.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Uric acid</td>
<td>198</td>
<td>1</td>
<td>0</td>
<td>31</td>
<td>195.1102</td>
<td>1.5</td>
<td>6.2</td>
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<tr>
<td>Creatinine</td>
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<td>0</td>
<td>31</td>
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<td>6.2</td>
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**Table 2**

**LC-MS/NMR**

**Results Standard Mixture**

**LUMC & Brucker Daltonics**

**GC/APCI with ultra high resolution TOF-MS: analytical validation and applicability to metabolic profiling**

Tiziano Petruzzelli1, Angela Carasco2, Emanuele Raimondi3, Tiziana Mantella4, Sandra Arosio5, Andrea M. Deelder6, Hartmut Schäfer7, Andrea A. Dokter1, DFG, Germany

**Introduction**

Most of the potential NMR metabolite systems are based on positive ionization mode coupled to high-resolution TOF-MS in order to overcome problems associated with low sensitivity and interference by unknown compounds. The main advantage of the TOF-MS is its ability to separate ions of different masses and to measure the mass to charge ratio (m/z) of the molecules. In this study, the combined use of GC and TOF-MS allows high-resolution measurements, reproducibility, and specificity at low costs. In the present study, the reproducibility of the platform was measured using a standard mixture of compounds, and the results were compared with those obtained using LC-MS and NMR.

**Methodology**

The platform features a liquid handler (MCI), a liquid nitrogen tank, and a liquid chromatograph (Bruker Daltonics).

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Metabolomics Data evaluation, Identification of unknowns

**Introduction**

An essential component on the process of characterizing chemical unknowns, is the inspection of collision induced disassociation (CID). Mass spectra from quadrupole/time-of-flight (Q-TOF) instruments. The high resolution and superior mass accuracy is key to establish the elemental composition of molecules. Unfortunately with increasing MS/MS spectra, the number of possible precursor formulae increases. Two different approaches are generally used for interpretation methods. First, theoretical fragmentation pathways are implemented using a novel software module which is able to predict all possible precursor formulae. Second, a novel software module which is able to predict all possible precursor formulae.

**Experimental**

- **Setup.** 10 μl of standards (1-10 μg/μl).
  - Mass spectrometer: nanoESI-TOF, Bruker Daltonics.
  - Capillaries: 100 μg.
  - Solvents: 100 μg.
  - Mass spectra: MS (ions), MS/MS (ions), and MS/MS (m/z).
  - MS/MS collision energy: 4-10 eV.

**Algorithm**

The elucidation multiple precursor formulae and corresponding product ion formulae is performed using the following steps:

1. Generate all possible precursor formulae for the precursor ions, as well as for all product ions, applying rigorous filters to remove ions which fail mass accuracy, formula inaccuracy, ratio of theoretical and observed masses, and higher mass transitions.
2. Filter out all product ion formulae which are not a subset of the precursor ion formulae. Connect every optimal pair with neutral loss candidates. Eliminate all formulae that belong to a higher mass transition.
3. The final set of product ion formulae, with loss mass for each precursor ion, product ion, and neutral loss.
4. If high MS/MS data are available, they can be used for reducing the sets of possible candidate formulae.

**Conclusions**

- ESI-Qq-TOF mass spectrometers provide accurate MS, MS/MS and ESI-Qq-TOF data are important tools in structure elucidation.
- The isotopic pattern plays an important role in the generation of formulas for MS to MS spectra.
- The automated generation and consolidation of proposed formulas for observed ions and neutral losses increases the confidence in resulting formulas for precursor and product ions.
- This significantly accelerates the interpretation of MS and MS/MS spectra.

---

**Table 1.** Compound with annotation of losses derived from isCID spectra.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass (m/z)</th>
<th>Error (ppm)</th>
<th>Loss (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>359.0989</td>
<td>±25.0</td>
<td>174.0610</td>
</tr>
<tr>
<td>BPE of pollen extract</td>
<td>509.2170</td>
<td>±2.0</td>
<td>259.1081</td>
</tr>
<tr>
<td>EIE</td>
<td>443.0989</td>
<td>±25.0</td>
<td>288.0791</td>
</tr>
</tbody>
</table>

---

**Fig. 2:** Measured and simulated pattern information has been added to MS/MS and isCID spectra.

---

**Fig. 3:** Measured and simulated pattern information has been added to MS/MS and isCID spectra.

---

**Scheme 1:** Fragmentation pathway of 1. Blue, red ions have been subjected to fragmentation (H2O, MS and isCID-MS).

---

**References**

**Introduction**

Phenolic compounds have great importance in the nutrition, organismic and health aspects of fruits and beverages. Furthermore, their consumption has been associated with health benefit such as antioxidant, anti-inflammatory, anti-carcinogenic, and antiviral properties. The total amount of phenolics depends on the anthocyanin content and its biological properties. A method for the rapid and reliable identification of phenolic compounds in foods based on accurate mass and library search has been developed.

**Methods**

**Chromatographic separation.** HPLC Agilent 1100 LC/TOF-MS (Agilent Technologies, Santa Clara, CA) using a 5-µm H/C ratio and 556.3567.

<table>
<thead>
<tr>
<th>Sample injection volume</th>
<th>Mobile phase</th>
<th>Gradient:</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 µl</td>
<td>acetonitrile-water (95:5)</td>
<td>0-3 min (0.0%) → 100% (0.0%) → 100% (19%) → 5% (20%) → 5% (21%)</td>
</tr>
</tbody>
</table>

**Scan range:** 50–800 m/z. 
**Scan mode:** positive and negative concomitant. 
**Mass accuracy:** 0.5 ppm.

**Library generation.** MS and MS/MS mass spectra of 1100 candidates were acquired and stored in the Bruker Daltonics library. MS/MS spectra of 1100 candidates were acquired and stored in the Bruker Daltonics library.

**Library analysis.** Candidates were calculated using the 5 ppm mass accuracy information into account. Only one of the five sum formulae could be found in the library database (see Chemspider), using the compound and library search method.

**Results**

- Full-scan mass spectra on the MicrOTOF Q III are given as exact mass value from 1100 to 1500 for the functional metabolites with a mass accuracy of 0.5 ppm. 

**Conclusions**

- A library of dietary phenolic compounds was successfully created using data acquired with a microTOF-Q III mass-spectrometer.
- The supported LC-AutoHEN/MS search method was fast, sensitive, and the identification accuracy of the selected sum formulae, and provides mass accuracy information into account.
- Full-scan mass spectra were obtained in a variety of samples analyzed under the same conditions from those of the reference spectra.

![Image](image1.png)

**Fig. 1** Histogram of parent ion mass spectra for the selected sum formulae.

**Fig. 2** Library search results for the sum formulae absolutely determined with a mass accuracy of 5 ppm.

The additional peptide signal of the compounds with a mass accuracy of 5 ppm was used to support the identification of the selected sum formulae.

**Fig. 3** Examples of the selected sum formulae.

An additional peptide signal of the compounds with a mass accuracy of 5 ppm was used to support the identification of the selected sum formulae. Only one of the five sum formulae could be found in the library database (see Chemspider), using the compound and library search method.
Deconvolution and Automatic Formula Assignment of Alternating MS and Broad-Band CID Analyses

Peter Sander, Dietmar Krohe, Sebastian Gürt, and Brigitte Schröder, Also Banck
Bruker Daltonics GmbH, Bremen, Germany

Introduction
Typical metabolite profiling applications are found with complex samples: partly overlapping characteristic masses at corresponding retention times makes it impossible to assign a confident structure to a signal in the spectrum.

Alternating MS and broad-band CID provides a possibility of deconvoluting the information from each scan and revealing the underlying signals. Here, we apply an automated workflow for the deconvolution and assignment of fragment ions resulting from a tandem mass spectrometry scan.

Detection of physiological amounts of neurotransmitters in complex matrices is important for understanding disease mechanisms, such as Parkinson’s disease. In this study, we demonstrate the ability to detect these molecules in rat brain tissue images using MALDI Imaging.

Methods
MALDI Imaging was performed using a Bruker Smartbeam II laser microprobe focusing onto a spot size of 30 µm and a laser repetition rate of 100 Hz. A Spectrin laser line at 355 nm was used as excitation wavelength. The mass spectra were acquired in positive ion mode using a TOF/TOF mass spectrometer.

Results
Bruker Daltonics software was used for data analysis. The data was acquired in PTP mode and the data was deconvoluted using the software’s inbuilt deconvolution algorithms. The peak lists were then used to calculate the elemental composition of the detected compounds.

Conclusions
Automatic deconvolution of complex samples is possible using alternating MS and broad-band CID. The automated workflow is reliable and robust, and provides consistent results across different tissues and images.

Acknowledgements
This work was supported by the German Federal Ministry of Education and Research (BMBF) under grant 0313656A. We would like to thank Dr. Dietmar Krohe for his support and advice throughout this project.

Literature

Small Molecule Imaging

Small Molecule Imaging Mass Spectrometry using the solariX-MI

Jern Fischer and Michael Becker
Bruker Daltonics GmbH, Bremen, Germany

Introduction
Image analysis of small molecules in tissue with MALDI TOF mass spectrometry requires the acquisition of a series of MS spectra for each target compound.

The acquisition of these spectra over the entire tissue sample is time-consuming. The high mass accuracy and mass resolution of FT-ICR MS allow an alternative single laser-microscope strategy for imaging small molecules in tissue. However, for MALDI imaging FT-ICR MS, internal calibration is necessary to establish an accurate mass scale. This is achieved by recording a set of ions with known masses, which are detected in the mass spectrum of the tissue.

During development, we demonstrated the ability to acquire MALDI imaging FT-ICR MS spectra with high mass accuracy and mass resolution.

Methods
Tissue samples were excited using a single crystal dye laser ( solariX-MI) equipped with an internal calibration. This allows the measurement of a large number of compounds in one tissue sample.

Results
MALDI Imaging MS spectra have been acquired with a solariX 10 FT-ICR mass spectrometer equipped with a Dual Resonance Ion Source with Induced Coupled Layer Mass Spectrometry. The data were acquired with a laser repetition rate of 100 Hz and a laser power of 100 mJ.

Conclusions
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