Analytical methods and algorithmic tools for liquid chromatography coupled to Orbitrap mass spectrometry

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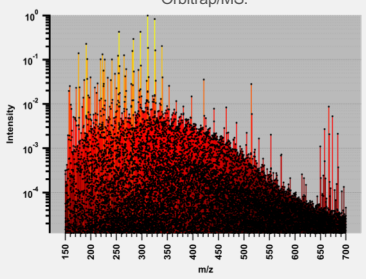
Context

Figure 1: Direct Infusion of Murchison in ESI(-)-Orbitrap/MS.



Very high resolution mass spectrometry is an analytical technique that can unveil the chemical diversity out of every samples, even complex ones. In the near future, several missions will bring back extraterrestrial matter from diverse environments that will be analyzed using state-of-the-art techniques, including very high-resolution mass spectrometers. Then, the CosmOrbitrap, a very high-resolution mass spectrometer has been developed and proposed for future space missions. Its capability to perform very high resolution mass spectrometry for a wide range of extraterrestrial environment, from icy satellites atmospheres and surfaces to comets and asteroids will bring us access to their chemical complexity *in situ*.

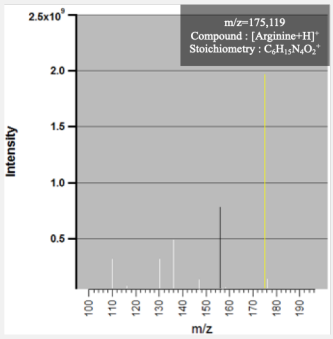
Several dedicated experimental protocols and algorithmic tools have been developed at the "Institut de Planétologie et d'Astrophysique de Grenoble" for the past decade, and this work now add chromatography and it's data treatment tools for biomarkers identification to the panel of methods and tools used in Grenoble laboratory. These methods are applied to extraterrestrial material (meteorites) and astrophysical analogues, and can also be applied to Hayabusa 2 sample return analysis or other space missions.

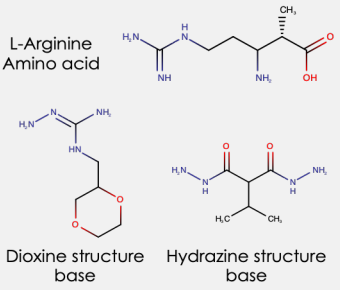
Analytical Workflow Description

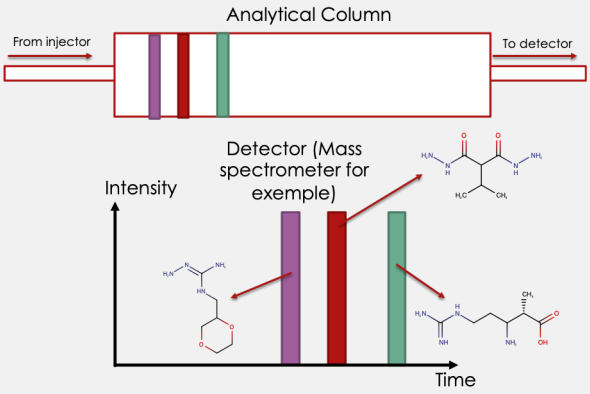
We developed a complete analytical workflow based on two steps: (1) a direct infusion acquisition and (2) a liquid chromatography analytical workflow. The required data treatment is supported by Attributor, an home-made software. This workflow is applied to meteorites and several analogues for a deep characterisation of the detected species structures. Having a standardized procedure will help compare samples between them, even from different days acquisitions.

Molecules by molecular formula "C6H14N4O2"

There are 125 commercially available compounds with a molecular formula of "C6H14N4O2" in MolPort database.

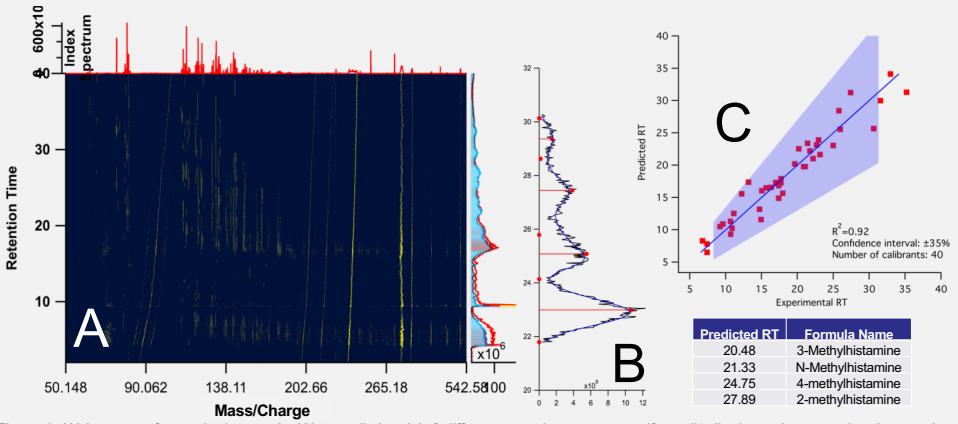






Mass Spectrometry: access stoichiometric formulas inside sample

Chromatography: access structures inside sample



Predicted RT	Formula Name
20.48	3-Methylhistamine
21.33	N-Methylhistamine
24.75	4-methylhistamine
27.89	2-methylhistamine

Figure 4: (A) Ion map of a synthetic sample. We can distinguish 2 different retention zones: a uniform distribution at low retention time, and an hyperbolic distribution at higher retention times. (B) Chromatogram extract of the ion m/z=126.102u (C6H12N3+(2.2ppm error), Methylhistamine annotated) that shows, after peak detection and peak-fitting, the separation of 4 isomers (RT(min): 22.8 ; 24.6 ; 27.2 and 29.4). (C) Prediction time model built with 40 calibrants and 10 physico-chemical coefficients. This model allows the prediction of the retention time of more than 2000 compounds in the biochemical database. The predicted retention time for the m/z=126.102u shows that the experimental RT=29.4min cannot be explained by the compounds in the data base.

A good chromatographic condition for the separation and identification of biochemical compounds is an HILIC column. To have a better identification of the compounds, having several methods with complementary information will restrict the solutions possibilities. The HILIC column run at acidic and basic pH have this complementarity, enabling the design of a unique analytical workflow using only one column.

The ion map and peak-fitting features (Figure 4A and 4B) are the results of the home-made software chromatographic data-treatment Attributor.

The core of the treatment are the detection and fitting of the peaks. After peak fitting, retention time prediction is done (Figure 4C) to do the identification of the molecules based on their structure. The retention time prediction principle is adapted from the work of [1].

Conclusion

The proposed analytical workflow is able to: (1) annotate molecules based on their stoichiometric formulas, (2) separate molecules based on their physico-chemical properties, (3) predict retention time based on physico-chemical properties of the molecules and (4) confirm or exclude separated molecular structures based on the comparison between the experimental and predicted retention time.

This workflow has to be applied to meteorites and relevant analogues to compare their shared molecules and then designate some synthesis conditions that can be at the origin of the meteorite organic matter diversity. This workflow can also be used for the Hayabusa 2 and CosmOrbitrap experiments to help explain the molecular features that are detected.

Références : [1] Creek DJ, et al. (2011) Analytical Chemistry 83(22):8703–8710.

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