**ORBITRAP-MS AND CHROMATOGRAPHY IN PREPARATION FOR HAYABUSA2 MOLECULAR COMPLEXITY ANALYSES.**

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**Introduction:** High-resolution mass spectometry (HRMS) is a powerful tool for unraveling the molecular content of mixtures[1]. Chromatography on its side is unmatched for molecular identification. Both have helped with the diversity and the polymeric structure of soluble organic matter in meteorites and laboratory analogs[2,3]. The HRMS indeed is able to detect each redundant mass differences and group molecules by polymeric families. The chemical separation based on the retention in chromatographic columns helps identifying isomers that can have completely different molecular structures one to another. Therefore, the combination of the two allows to tell if the synthesis patterns and the final composition are of one single process or if there is a complex history at the origin of a given mixture.

For instance, the evolution of the average aliphatic chains length can be related to growing of destructing processes. Given homologous families (one common molecular structure with one or several aliphatic parts of variable length) have already been described in carbonaceous meteorites, the variability of the chains length is a test for the co-synthesis of the whole mixture[4].

The use of this techniques for priceless samples like regolith from the surface of Ryugu is high risk for high reward, with definitive answer on the distribution of organic molecules in the solar system. Here we present preliminary results concerning the rehearsal measurements done on Murchison with a LC-HRMS instrument.

**Methods:** The goal is to compare data from macro-sample, here grams of Murchison to micro-sample. Few milligrams of Murchison were crushed manually and macerated in different solvents sequentially from apolar (dichloromethane) to polar (methanol). The extracts were then infused in a C-18 reversed phase column with a classical acetonitrile to water elution gradient. Both for macro and micro sample, a minimal consumption Nano-LC pump was used in line with an Exactive-Orbitrap mass spectrometer. High resolution mass spectra were acquired every ~1 seconds for 40 minutes at Kyushu University. This type of column and gradient are designed to separate mixtures by the polarity of their components. This strategy suits the Murchison mixture in the sense the dominant variability is the chain length which will control the interaction with the column and determine the retention time of each compound.

Data analysis were done using the ATTRIBUTOR house made software to represent and evaluate the mass distributions, ions formulas and their retention time. It provides a quick overview of the chromatographic behavior of the mixture through the so-called ion map, plotting the abundance (color coded) of each peak in the retention time/mass space (fig.1). For any domain of this ion map, the classical tools for HRMS are available such the Mass Defect vs Mass analysis and the stoichiometric network. New development on the automatic peak classification will be shown and used to ease the interpretation.
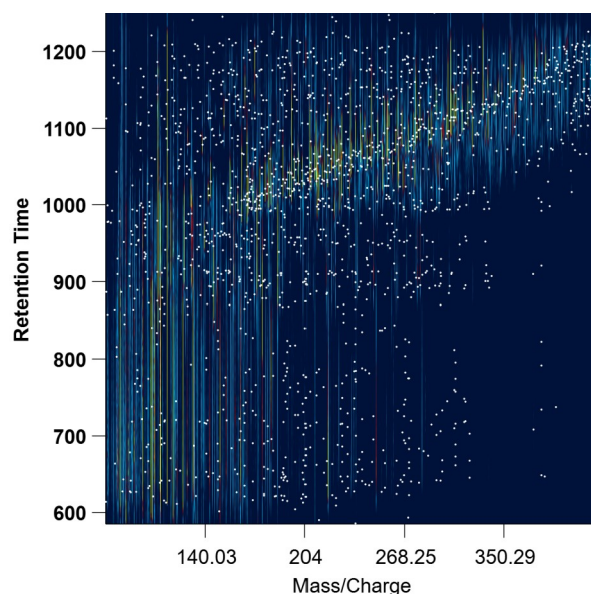


Figure 1: Ion map of the micro-sample of soluble in methanol Murchison extract. The denser part with retention time above 1000 seconds shows families of variable chain length, the latter being responsible of the correlation between polarity (increase of retention time) and mass.

**Results:** For the macro sample, dichloromethane (DCM), methanol and water extracts are available for Murchison meteorite. DCM and methanol extracts have different yet comparable chromatograms. As

expected, the column separates molecules by polarity. Two major groups are observed and can attributed to O-rich and O-poor distributions. The water extract has a very different chromatogram were the column separation is moderate. In the case of extreme polarity, the hydrophobic column may not be the best choice to investigate chemical diversity. Another type of column is considered to go further in highly polar extracts.

For the microsample of Murchison, only the methanol extract is available for comparison and more details will be discussed as the data acquisition is complete.

The figure 1 shows the ion map associated with the methanol extract and clearly shows several groups of compounds. The one with the lowest retention times (below 1000 seconds) are related to the compounds poorly retained by the hydrophobic column and therefore interpreted as the most polar molecules. They indeed exhibit typical negative mass defect patterns identified in direct infusion spectra. This is consistent with higher amount of oxygen bearing functions. They are of lower abundance compared to the other group with higher retention times (above 1000 seconds).

Indeed, the group corresponds to the less polar compounds and exhibit a correlation between the retention time and the mass/charge. This is interpreted as the chain length variability identified in direct infusion spectra. This shows that the different families span a common mass and retention time (polarity) range.

The $CH_2$ distributions found in the bulk, direct infusion, spectrum are present and can be compared from one retention time to another. This tends to prove that retention time is controlled by the aliphatic chains variability, more than the heteroatomic functions attached to. Further investigations on the fine distribution of the $CH_2$ patterns will tell if the molecular growth process is the same from one homologous family to another.

**References:** [1] Schmitt-Kopplin, et al. (2010). *PNAS* 107, 7: 2763–2768. [2] Naraoka, et al. (2017). *ACS Earth and Space Chemistry* 1, 9: 540–550. [3] Yamashita and Naraoka. (2014). *Geochemical Journal* 48, 6: 519–525. [4] Orthous-Daunay, Piani, Flandinet, et al. (2019). *Geochemical Journal* 53, 1: 21–32.