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METABOLITES IDENTIFICATION IN NON-TARGETED METABOLOMICS APPROACHES: A MULTI-ANALYTICAL MASS SPECTROMETRY PLATFORM VIEW

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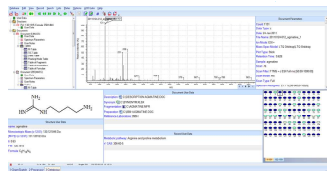
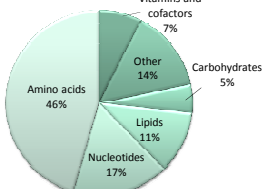
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Introduction

Non-targeted metabolomics approaches include various steps, among these, structural elucidation of metabolites of interest still remains important bottleneck [1]. To facilitate metabolite identification, an in-house-LC-²HRMS database containing information about chromatographic retention time, accurate mass and fragmentation pattern, using ACD/Labs software, has been developed [2]. In addition, an automatic Excel[®] macro to help filtering and allowing a fast and direct annotation of compounds in the XCMS table has been elaborated. Nevertheless, these criteria are not always sufficient for unequivocal identification being the use of other analytical and complementary techniques necessary. As a result, a multi-analytical platform based on both LC-²HRMS using the complementarities of reversed phase and HILIC separations, and GC-MS has been developed for a comprehensive metabolome and lipidome characterization, coupled to an in-house reference database [3].

Metabolites identification & automatic annotation

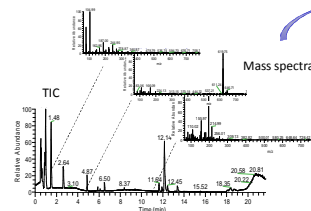
✓ LC-²HRMS and MS/MS metabolomics in-house database



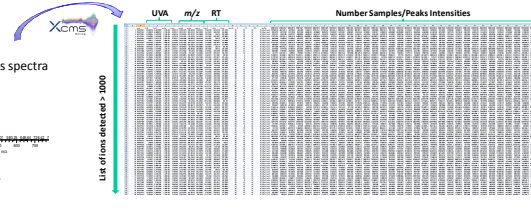
Main information stored :
Specific RT ✓
HRMS Spectra (AM) ✓
MS/MS Spectra ✓

- Reversed phase LC
- ESI+ and ESI-
- > 250 metabolites
- Citrate metabolism, glycolysis, purine and pyrimidine metabolism,...

✓ Global strategy



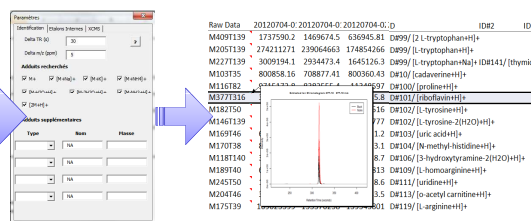
Data processing



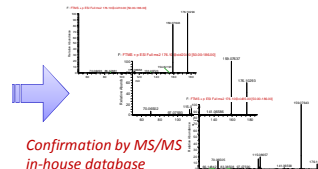
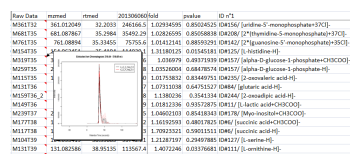
Excel macro
Metabolites ID: AM and RT
Automatic annotation

✓ Automated Excel[®] macro

To allow a fast and automatic annotation of compounds, a specific Visual Basic script was developed to be run in the Excel[®] software. The basic idea of this algorithm is to compare experimental *m/z* and RT obtained after the data processing using XCMS with the reference values implemented in the database. In addition, possible adduct ions are also considered improving metabolite ID.

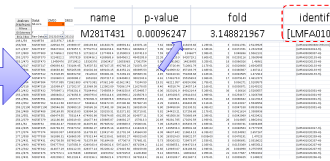
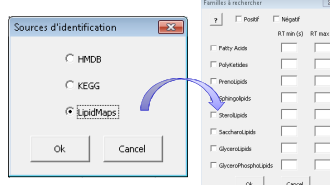


□ In-house Database ID:

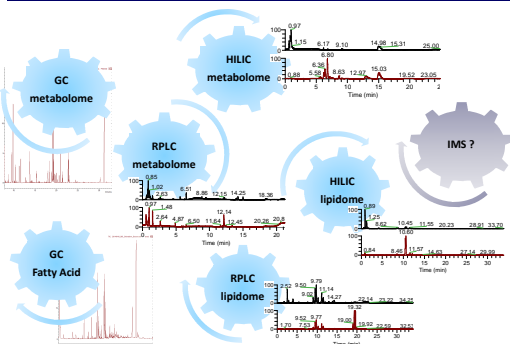


Confirmation by MS/MS in-house database

□ Additional links toward external online databases (HMDB, KEGG and LIPID MAPS):



Multi-analytical platform



- RPLC-²HRMS metabolome**: Polar metabolites
- HILIC-²HRMS metabolome**: Highly polar metabolites
- GC-MS metabolome**: Polar metabolites such as sugars,...
- GC-MS Fatty Acid**: Targeted analysis of fatty acids
- RPLC-²HRMS lipidome**: Alkyl chain interaction → Lipids speciation
- HILIC-²HRMS lipidome**: Head group interaction → Lipid classes separation
- IMS-²HRMS**: New dimension for metabolomic? ✓ IMS: Separation and identification through ion mobility in gas phase (isomers, conformers, ...).

- ✓ The use of RP and HILIC chromatography allowed to obtain complementary chromatographic separations for metabolomics approaches and analysis of complex lipids.
- ✓ GC-MS makes enable the detection of specific metabolites generally not detected by LC-MS. Furthermore an orthogonal identification of metabolites detected by LC-MS is achieved.
- ✓ Possibility of reactive database update according to a novel or modified analytical workflow.

Conclusion

Correct assignment of chemical identities to signals generated by MS based metabolomic approaches is a pre-requisite for any scientific outcome. Therefore, raw data have to be correctly processed through properly developed scripts and identification of revealed markers must become high-throughput. Indeed, the development of an experimental spectral database greatly facilitates metabolite identification based on retention time, accurate mass and fragmentation pattern. However, this spectral database will be continuously completed to increase the number of metabolites. On the other hand, the use of a multi-analytical MS platform clearly enlarge the number of metabolites detected and improve their identification. All approaches are supervised under the quality management system set up in laboratory.



References:
[1] Cresk, D.J., et al., *Metabolomics*, 2014, 350-353.
[2] Courant, F., et al., *Analyst*, 2012, 4958-4967.
[3] C. Jacob, C., et al. *Metabolomics*, 2015, 184-197.