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

A.-L. ROYER, H. GALLART-AYALA, S. CHEREAU, F. MONTEAU, G. DERVILLY-PINEL, J.-P. ANTIGNAC, B. LE BIZEC



Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA)
USC INRA 1329, Oniris, LUNAM Université, BP 50707, 44307 Nantes Cedex 3, France
Fax : +33 (0)2 40 68 78 78 - Tél : +33 (0)2 40 68 78 80
laberca@oniris-nantes.fr

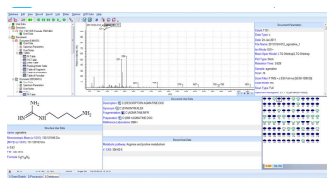
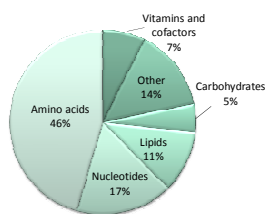


Introduction

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-HRMS database containing information about chromatographic retention time, elemental composition 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 established. 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-HRMS using the complementarities of reversed phase and HILIC separations and GC-MS has been developed for a comprehensive metabolome and lipidome characterization.

Metabolites identification & automatic annotation

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

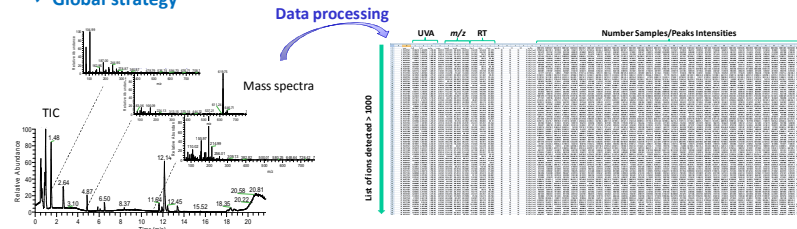


Main information stored :

- Specific RT ✓
- HRMS Spectra (AM) ✓
- MS/MS Spectra ✓

- > 240 metabolites
- Reversed phase LC
- ESI+ and ESI-

✓ Global strategy



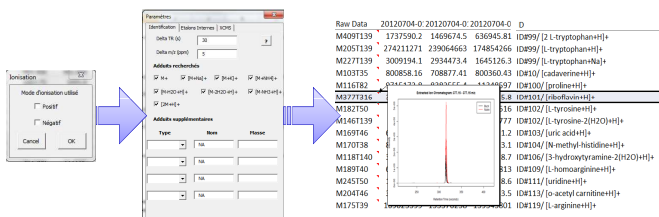
Data processing

Excel macro

Metabolites ID: AM and RT
Automatic annotation

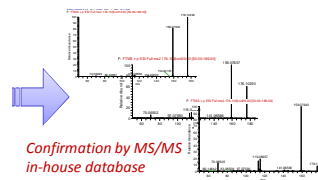
✓ Automated Excel® macro

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.

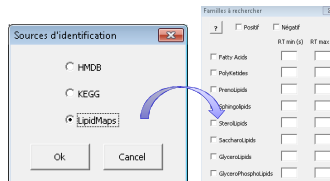


□ In-house Database ID:

Raw Data	name	rt	rt	201300000000	rt	rt	ID#1
M08112	341.02309	32.2033	248364.5	1.02949595	0.8004425	0.04156	[Lysine-5'-monophosphate-3PC]
M08115	445.00767	31.2884	34941.29	1.02929595	0.8004958	0.04156	[Phenylethylammonium-3PC]
M07135	761.08834	35.33455	79758.6	1.0442141	0.88951291	0.04156	[Guarantonic-3'-monophosphate-15SC]
M04155	101.00000	10.00000	101.00000	1.0000000	0.0000000	0.00000	[L-malic acid-H]
M13175	1.038879	0.8931939	0.04157	0.04156	0.04156	0.04156	[alpha-D-glucose-5-phosphate-CH2COO]
M05175	1.0573832	0.8848195	0.04157	0.04156	0.04156	0.04156	[D-serine-H]
M15175	1.0713208	0.8671517	0.04157	0.04156	0.04156	0.04156	[glutamic acid-H]
M01716	1.1389236	0.9341334	0.04156	0.04156	0.04156	0.04156	[D-malic acid-H]
M08716	1.01812336	0.89572875	0.04157	0.04156	0.04156	0.04156	[lactic acid-CH2COO]
M02177	1.00602333	0.80483433	0.04156	0.04156	0.04156	0.04156	[beta-D-glucose-5-phosphate-CH2COO]
M07718	1.14182539	0.88027825	0.04156	0.04156	0.04156	0.04156	[succinic acid-CH2COO]
M01178	1.17012517	0.90955512	0.04156	0.04156	0.04156	0.04156	[succinic acid-H]
M04179	1.12181797	0.79487885	0.04157	0.04156	0.04156	0.04156	[L-serine-H]
M01179	1.11826286	0.8391515	0.04156	0.04156	0.04156	0.04156	[L-arginine-H]

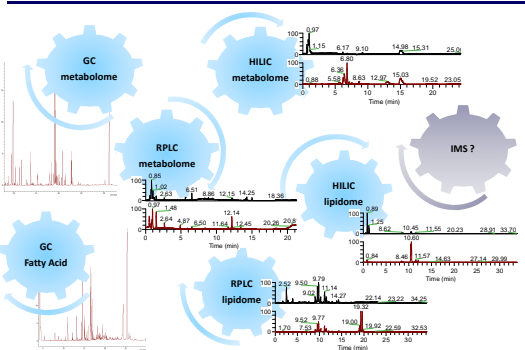


□ Other databases ID (HMDB, KEGG and LIPID MAPS):



name	p-value	fold	identification
M281T431	0.00096247	3.148821967	[LMFA01020206-H]

Multi-analytical platform



- RPLC-HRMS metabolome** Polar metabolites
 - HILIC-HRMS metabolome** Highly polar metabolites
 - GC-MS metabolome** Polar metabolites such as sugars,...
 - GC-MS Fatty Acid** Targeted analysis of fatty acids
 - RPLC-HRMS lipidome** Alkyl chain interaction → Lipids speciation
 - HILIC-HRMS lipidome** Head group interaction → Lipid classes separation
 - IMS - HRMS** New dimension for metabolomic? ✓ IMS : Separation and identification of ionized molecules 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.

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.