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ITERATIVE DDA LC-MS/MS METHOD AND MOLECULAR NETWORKING

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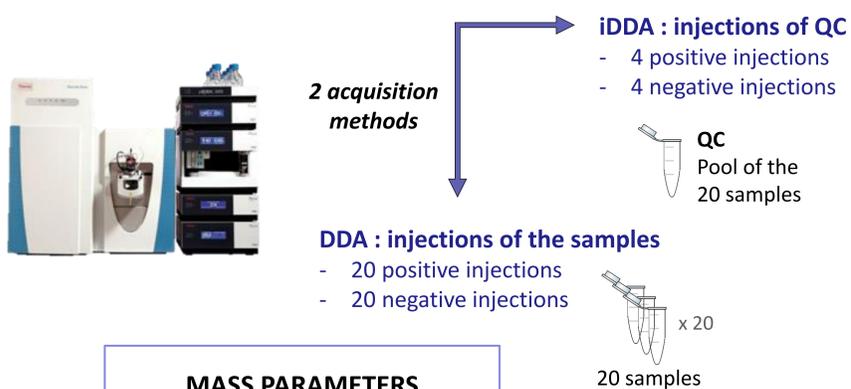


Introduction

The annotation step probably is one of the most difficult part in the metabolomics workflow. To expand the number of features annotated, an iterative Data Dependant Acquisition (iDDA) method was applied on an LC-Orbitrap, based on Jeremy Koemel's paper (*Expanding Lipidome Coverage Using LC-MS/MS Data-Dependent Acquisition with Automated Exclusion List Generation* <https://doi.org/10.1007/s13361-017-1608-0>). The number of features obtained with this method from the Quality Control sample is compared with a single DDA method on all the samples, using the online Workflow4Metabolomics infrastructure (<https://workflow4metabolomics.usegalaxy.fr>) for annotation pipeline and MetGEM (<https://metgem.github.io/>) for molecular network's construction.

Experimental Workflow

1 Acquisition LC-Orbitrap (Thermo)



MASS PARAMETERS

Full MS

- Resolution = 35,000
- AGC Target = 1^ε6
- Maximum IT = 100 ms

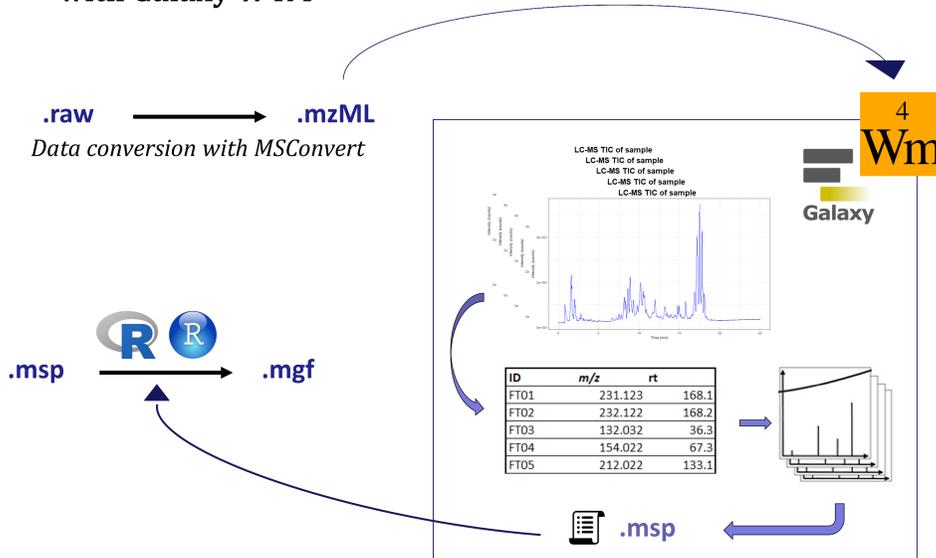
ddMS²

- Resolution = 17,500
- Top N = 5
- Isolation window = 1.0 m/z
- Dynamic exclusion = 3.0 s

+ Exclusion list iDDA method



2 Integration, Alignment, Deconvolution with Galaxy W4M



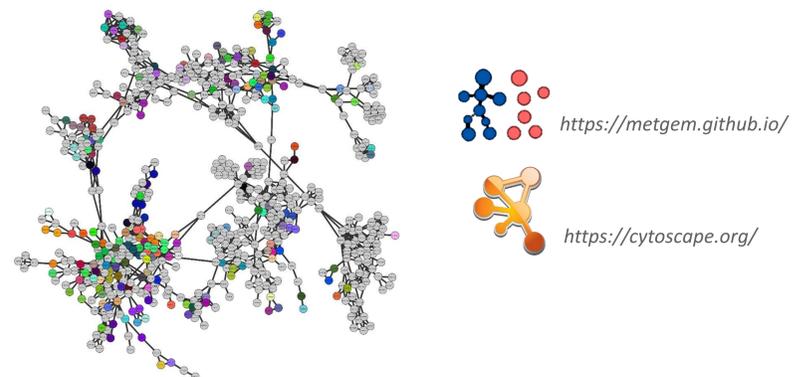
3 Annotation with databases Level 3 of Schymanski's annotation scale



Level 1 : Confirmed structure by reference standard	MS, MS2, RT, Ref Std, CCS
Level 2 : Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS2, Library MS2 MS, MS2, Exp. Data
Level 3 : Tentative candidate(s) structure, substituent, class	MS, MS2, Exp. Data
Level 4 : Unequivocal molecular formula	MS isotope/adduct
Level 5 : Exact mass of interest	MS

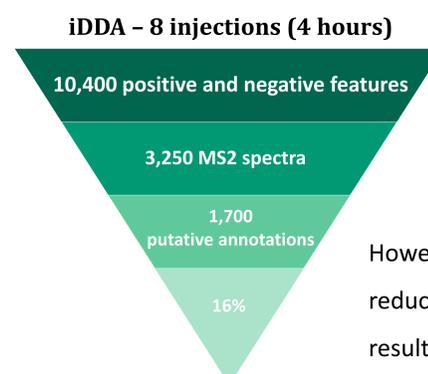
<https://doi.org/10.1021/es5002105>

4 Molecular Network with MetGem & Cytoscape



Results and discussion

The iDDA method allows to reduce injection time by 5 and the annotation ratio is identical to the DDA method (16%).



DDA - 40 injections (20 hours)

18,000 positive and negative features

5,000 MS2 spectra

3,100 putative annotations

17%

iDDA - 8 injections (4 hours)

10,400 positive and negative features

3,250 MS2 spectra

1,700 putative annotations

16%

However, the number of putative annotations is reduced by half and it is not consistent with the results obtained in Koemel's paper.

Conclusion

Simple DDA on each sample is time consuming. Using iDDA on a pool of the samples should lead to a lot more informations with less injections and so, shorter time of analysis.

The annotation workflow with Galaxy and MetGem works well but mass parameters for DDA and iDDA method need to be optimized. The other idea to improve this iDDA method is to use an in silico tool (<https://doi.org/10.1021/acs.analchem.0c03895>) which allows to optimise the DDA parameters from real samples.