



Master Thesis | Plant Metabolomics Laboratory (ITQB-UNL)

Title: Target metabolomics approaches to study plant responses to abiotic stress

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Duration: 9 to 12 Months

Abstract: Reversed phase (RP) chromatography is an established approach in mass spectrometry-based metabolomics. However, many primary metabolites, in particular carbohydrates, are highly polar compounds, and show minimal retention on typical RP stationary phases therefore eluting very close to the void volume without chromatographic separation. Normal phase separations have traditionally suffered from poor reproducibility and separation efficiencies and tend to use eluents that are not readily compatible with mass spectrometry. We have previously developed robust alternative online LC-ESI-MS/MS methods using:

- (i) Porous graphitic carbon (PGC) stationary phases for the simultaneous targeted analysis of several intermediates of the glycolytic metabolic pathway from *A. thaliana* leaf material [1], and to detect small alterations in carbohydrate levels under drought stress conditions in the stems of *Lupinus albus* [2] and in the leaves of the resurrection plant *Haberlea rhodopensis* [3].
- (ii) Hydrophilic interaction liquid chromatography (HILIC) stationary phases for the simultaneous targeted analysis of neutral and charged plant primary metabolites [4].

The project consists in using several metabolomics methodologies, from sample preparation for metabolomics analyses to the extraction of primary metabolites and LC-MS/MS target analysis to simultaneously separate and identify key stress-responsive metabolites (osmolytes) that accumulate in plants under abiotic stress conditions [6].

References:

- [1] Antonio C, Larson T, Gilday A, Graham I, Bergström E, Thomas-Oates J (2007) Quantification of sugars and sugar phosphates in Arabidopsis thaliana tissues using porous graphitic carbon liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr A* 1172: 170-178.
- [2] Antonio C, Pinheiro C, Chaves MM, Ricardo CP, Ortuño MF, Thomas-Oates J (2008) Analysis of carbohydrates in *Lupinus albus* stems using porous graphitic carbon liquid chromatography-electrospray ionization mass spectrometry, on imposition of water deficit. *J Chromatogr A* 1187: 111-118.
- [3] Gechev T, Benina M, Obata T, Tohge T, Sujeeth N, Minkov I, Hille J, Temanni R, Marriott AS, Thomas-Oates J, Antonio C, Mueller-Roeber B, Schippers JHM, Fernie A, Toneva V (2013) Molecular mechanisms of desiccation tolerance in the resurrection glacial relic *Haberlea rhodopensis*. *Cell Mol Life Sci* 70: 689-709.
- [4] Antonio C, Larson T, Gilday A, Graham I, Bergström E, Thomas-Oates J (2008) Hydrophilic interaction liquid chromatography-electrospray ionization mass spectrometry analysis of carbohydrate-related metabolites in Arabidopsis thaliana leaf tissue. *Rapid Comm Mass Spectrom* 22: 1399-1407.
- [5] Cubbon S, Antonio C, Wilson J, Thomas-Oates J (2010) Metabolomic Applications of HILIC-LC-MS. *Mass Spectrom Rev* 29: 671-684.
- [6] Jorge TF, Rodrigues JA, Caldana C, Schmidt R, van Dongen J, Thomas-Oates J, Antonio C (2015) Mass Spectrometry-Based Plant Metabolomics: Metabolite Responses to Abiotic Stress. *Mass Spectrom Rev* (<http://onlinelibrary.wiley.com/doi/10.1002/mas.21449/abstract>).

For further information, please visit our **Plant Metabolomics Laboratory** websites:

[1] <http://www.itqb.unl.pt/research/plant-sciences/plant-metabolomics/plant-metabolomics>

[2] <http://www.itqb.unl.pt/labs/plant-metabolomics>