

# LC-MS combined with sensitive CapNMR methods for natural product dereplication and biomarker identification in plant metabolomics

Jean-Luc Wolfender<sup>1</sup>, Gaetan Glauser<sup>1,2</sup>, Elia Grata<sup>1,2</sup>, Julien Boccard<sup>2,3</sup>, Pierre-Alain Carrupt<sup>3</sup>, Serge Rudaz<sup>2</sup>

<sup>1</sup>LPP, <sup>2</sup>LCAP, <sup>3</sup>LCT, School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, quai Ernest-Ansermet, CH-1211 Geneva, Switzerland.

The development of on-line LC-NMR and at-line methods (SPE-NMR and CapNMR) have open new exciting opportunities for the rapid identification of natural products (NP's) in the field of phytochemistry and plant metabolomics. The possibility to acquire rapidly <sup>1</sup>H-NMR spectra of individual constituents in crude plant extracts in direct hyphenation with HPLC provides useful complementary information to LC-UV-MS profiling and give a strategic advantage in the dereplication process. On the other hand the use of sensitive at-line methods based on microflow NMR such as CapNMR<sup>1</sup> in combination with LC-MS triggered microfractionation methods provides high quality 1D and 2D NMR spectra for the analysis of NP's in the low microgram range. Such experiments are indeed essential for a complete *de novo* structural determination. The acquisition of NMR data on sample scales equivalent to biological screening amounts can thus considerably accelerated the lead finding process or the identification of biomarkers in metabolomic studies.

In this respect different examples of plant analyses will be discussed. In particular the results obtained with UPLC-TOF-MS and CapNMR in the frame of a plant metabolomic study of the wound response in *Arabidopsis thaliana* will be presented.<sup>2</sup> In this case a non-targeted high throughput metabolite fingerprinting of numerous specimens involving rapid UPLC-TOF-MS analysis and data mining enabled the detection of different stress biomarkers such as oxylipins.<sup>3</sup> Key wound-induced jasmonate derivatives were isolated at the microgram scale by a precise MS-directed fractionation procedure for their complete *de novo* CapNMR structural determination. Upscaling from UPLC to semi-prep LC-MS relied on efficient gradient transfer and computed optimised chromatographic conditions with Osiris® software. A special attention was paid to the separation of closely related isomers.<sup>4</sup> Thanks to this strategy, a broad survey of wound-biomarkers with various physicochemical properties was obtained in the leaf extracts and, besides known signalling molecules, original oxylipins and related products were identified. A careful interpretation of the UPLC-TOF-MS data provides an overview of the spatial and temporal induction of the jasmonates.<sup>3</sup> The approach enabled both a rapid estimation of the significant wound metabolome variations and the precise identification of biomarkers involved in these changes. The biological activity of these products in relation with their defence gene expression potential was evaluated based on DNA microarray experiments.

- (1) Olson, D. L.; Norcross, J. A.; O'Neil-Johnson, M.; Molitor, P. F.; Detlefsen, D. J.; Wilson, A. G.; Peck, T. L. *Anal. Chem.* **2004**, *76*, 2966-2974.
- (2) Grata, E.; Boccard, J.; Guillarme, D.; Glauser, G.; Carrupt, P. A.; Farmer, E.; Wolfender, J. L.; Rudaz, S. *J. Chromatogr. B* **2008**, (*in press*)  
*doi:10.1016/j.jchromb.2008.04.021*.
- (3) Glauser, G.; Grata, E.; Dubugnon, L.; Rudaz, S.; Farmer, E.; Wolfender, J. L. *J. Biol. Chem.* **2008**, *283*, 16400-16407.
- (4) Glauser, G.; Guillarme, D.; Grata, E.; Boccard, J.; Thiocone, A.; Carrupt, P. A.; Veuthey, J. L.; Rudaz, S.; Wolfender, J. L. *J Chromatogr A* **2008**, *1180*, 90-98.