

## Hilic LC-MS: From targeted to untargeted

Narduzzi Luca, Arapitsas Panagiotis, Della Corte Anna, Angeli Andrea, and Mattivi Fulvio  
*Fondazione Edmund Mach, Research and Innovation Centre, Department Food Quality and Nutrition, via E. Mach 1, 38010 San Michele all'Adige, Italy*

Untargeted and targeted metabolomics approaches are sharing a similar fundamental workflow dividable in: a. extraction (sample preparation), b. separation or not (LC, GC, CE, direct infusion, etc), c. detection (MS and/or NMR), and d. data analysis (chemometrics).

Since targeted methods are used for many decades, their applications in all the above fields are further advanced and the knowledge for method development is well established. The global or holistic or untargeted techniques are rather new, and so there are still a lot of debates about the experimental design and validation. Actually, researchers develop their methods based in the experiences obtained by the targeted field. Most of the efforts are directed to the detection, mainly due to the late advantages of mass spectrometry; and to the data analysis, because datasets are very attractive for researcher working in high chemometrics, programming, and statistics; while sample preparation should always be the simplest possible. Up to now, the knowledge about separation part (especially Hilic methods) is poor.

In order to develop a holistic method, we took in consideration a targeted one, recently published for the quantitative profiling of 135 grape polar primary metabolites via hydrophilic interaction liquid chromatography (HILIC) UPLC-MS/MS (Gika et al 2012 JCA), and we adapted it for untargeted analysis. Since the targeted method was developed for the separation and quantification of a big number of polar metabolites belonging in various groups such as sugars, sugar alcohols, organic acids, bioamines, amino acids, etc; we decide to maintain the general chromatographic conditions (column, gradient, flow rate, column oven temperature, eluents and salt type and concentration of the eluents). Also the extraction protocol for the holistic analysis of grape polar metabolites was optimized lately (Theodoridis et al. 2012 Metabolomics). The studied parameters in this work, for the further LC part development, without losing any of its original advantages, were: 1. standardization of the eluents preparation, 2. sample dilution, 3. the number of injections needed for a new column equilibration before use, 4. and the number of injections for a good system stability. Most of these parameters are rarely taken in consideration while developing a targeted method, but are crucial for the repeatability, chromatographic alignment and stability of any untargeted method.