

*Title:* **Assessment of chromatographic separations and retention time predictions of polar metabolites**

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Metabolomics is an interdisciplinary scientific branch dedicated to the study of the metabolome, the set of low-molecular-weight molecules involved in the chemical reactions of all living creatures. The absence or presence of some metabolites, and their concentrations, enables the assessment of the state in which an organism, tissue or cell finds itself at a particular time. The obtention of this information, which can be extremely relevant from a pharmacological perspective and/or in research contexts, requires the development of analysis methods that detect and unambiguously identify as many metabolites as possible.

In this work, six liquid chromatography coupled to mass spectrometry (LC-MS) methods have been applied to the separation of mixtures containing amino acids, nucleosides and other metabolites, such as carbohydrates and short-chain organic acids. In order to ensure a proper resolution of the mixtures, which contained metabolites of varying polarity, the separations were performed by hydrophilic interaction liquid chromatography (HILIC). The chromatographer was coupled to an electrospray ionization (ESI) source and analytes were detected through MS using a time-of-flight (ToF) analyzer.

The obtained chromatograms were examined and compared through the calculation of two chromatographic response functions (CRFs) to determine which were the best elution conditions. Aiming to increase peak detection and assignment, models relating the annotated metabolites' physicochemical properties to their experimental retention times were generated. Then, the retention times of the unassigned metabolites were predicted. A careful inspection of the chromatograms, taking into account the predictions of the models, allowed for an increase in the detected and annotated metabolites, resulting into more of a 10 % augmentation in the total number of unequivocally assigned peaks.

**Keywords:** metabolites, LC-MS, HILIC-ESI/ToF, CRF, retention time prediction.