

Analysis of metabolites in ionic exchange chromatography coupled to mass spectrometry

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Over the years, number of diseases was diagnosed. In order to facilitate an early diagnosis and to propose an appropriate treatment, it becomes necessary to be able to detect and quantify quickly representative biomarkers of specific pathology. Among these biomarkers, we can find small acids as well as phosphate compounds associated to energy metabolism. These compounds are found in standardized concentrations in human body, these concentrations being influenced by the appearance and the development of a pathology ¹.

Several techniques are used to analyze these biomarkers, such as high-performance liquid chromatography coupled with mass spectrometry ² (HPLC-MS). These techniques require some sample preparation to avoid the signal of the matrix and simplify the analysis. In case of coupling with mass spectrometry, HILIC (hydrophilic interaction chromatography) is the most adapted technique in terms of compatibility with mobile phase but it presents some drawbacks. Indeed, HILIC stationary phases are well known to be less reproducible than reverse phase. Furthermore, retention times of these polar solutes are too short to separate a huge number of compounds in a complex matrix such as biological samples. In our project, an ion chromatographic tandem mass spectrometry method (IC-MS/MS) will be developed. For many years now, IC is considered as the method of choice concerning organic and inorganic anions analysis in various matrices. The presence of the suppressor in the device allows to minimize or remove the signal of the salted biological matrix like in urine and blood. Some methods have already been developed in IC to analyze some biological compounds without any sample preparation in urine or saliva ³.

In this work, IC-MS/MS is used for the energetic metabolism evaluation by the dosage of 52 molecules. These molecules are involved in different cycles such as glycolysis (pyruvate, lactate or glucose-6-phosphate), Krebs cycle (citrate, malate or acetate) or nucleotides catabolism (adenosine monophosphate, inosine monophosphate or urate). The first optimization of the method concerns the mass spectrometry parameters and identification of fragments obtained for each molecule. Following this, an optimization of IC parameters will be achieved in order to obtain an optimal separation of our compounds in one injection. This optimized method could be applied to detect and quantified these biomarkers in diluted biological samples.

¹ L.G. Baggetto, « Deviant energetic metabolism of glycolytic cancer cells », *Biochimie*, Vol. 74, **1992**, 959-974.

² P. Chen, « A LC-MS/MS method for the analysis of intracellular nucleoside triphosphate levels », *Pharmaceutical research*, Vol. 26, **2009**, 1504-1515.

³ L. Valentin-Blasini et al., « Analysis of perchlorate in human urine using ion chromatography and electrospray tandem mass spectrometry. », *Analytical Chemistry*, Vol. 77, **2005**, 2475-2481.