

METHOD DEVELOPMENT FOR THE DETECTION OF CARBOHYDRATES USING UPLC - TIMS - TOF (MS) TECHNIQUE. APPLICATION TO DIFFERENT TYPES OF HONEY**T. Zioga¹, A. Lioupi², C. Virgiliou^{1,3}**

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Carbohydrates, being among the most prevalent compounds, play a crucial role in sustaining life, providing energy and structural support. Glycomics research has unveiled additional roles, such as signaling and cellular recognition, highlighting the diverse importance of carbohydrates in biological processes, beyond their conventional functions^[1]. However, sugars, being isomeric compounds, pose challenges in their separation. Ion mobility spectrometry (IMS), an emerging gas-phase ion separation technology, has been considered as a promising tool for performing carbohydrate structure elucidation. The aim of the study is the development of a method for the determination and separation of isomeric carbohydrates using the high-resolution liquid chromatography technique hyphenated with ion mobility mass spectrometry UPLC-TIMS-TOF (MS). Separation of the analytes was performed on a hydrophilic interaction liquid chromatography (HILIC) stationary phase, by applying a 28 minutes gradient elution program. With the developed method we were able to separate and collect data including retention time, MS/MS spectra and CCS values for 23 carbohydrates. The developed method was applied in honey samples from three distinct flowers of origin, blossom, pine, and oak after optimization of sample preparation procedure. A total of 16 out of 23 sugars were detected in real samples, 10 sugars were found in floral honey, 12 sugars in pine honey, and 14 sugars in oak honey. Distinct differentiation was observed in the carbohydrate profiling of honey samples from different flower origin. Six sugars were commonly detected in all samples, while carbohydrates, such as trehalose and mannitol, were present in pine and oak and absent in honeydew honeys. In the present study we investigate the ability of TIMS-TOF instrumentation to differentiate the studied carbohydrates according to their structures and shapes and the usefulness of approach in several fields such as characterization of foodstuff samples and quality control, which remains a major challenge.

KEYWORDS: Ion mobility, HILIC, carbohydrates, UPLC-TIMS-TOF (MS), honey

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