ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Bc. Gabriela Šebestová

Supervisor: PharmDr. Pavel Jáč, Ph.D.

Title of the diploma thesis: Development of CE-C4D method for the analysis of saccharides in honey

Honey is a commonly used natural mixture which quality must be monitored due to possible mishandling or adulteration.

This diploma thesis is focused on the development of a capillary electrophoresis method with capacitively coupled contactless conductivity detection (CE-C^{4}D) for the determination of carbohydrates in honey samples. Design of Experiments (DoE) was used to optimize the separation conditions. This approach allows to find suitable separation conditions by means of multivariate optimization. During the optimization, the impact of the following factors was investigated: the composition of the background electrolyte, the separation voltage and temperature during separation and the inner diameter of the separation capillary. Two methods were optimized: one with normal electroosmotic flow (EOF) and the other using reversed EOF after CTAB addition.

The method in the normal EOF mode was used for validation and analysis of honey samples. A fused silica capillary with an inner diameter of 20 μm, a total length of 48.7 cm and an effective length of 33.8 cm was used for the separation. The temperature was 27.7 °C and the separation voltage was set at +16.5 kV. The background electrolyte composed of 35 mM NaOH and 15 mM Na$_2$HPO$_4$ was chosen as optimal. Galactose was used as the internal standard. Total analysis time was shorter than 5 minutes. The limit of quantification was 0.3 mM, what corresponds to a content of 5 g of sucrose per 100 g of honey allowing the determination of the maximum permitted amount of sucrose set by Decree of Ministry of Agriculture No. 76/2003 Coll. Linearity was verified in the range of 0.3 mM to 7.5 mM with correlation coefficients higher than 0.9984 for all analytes. All three honey samples analyzed by this method met the requirements defined by the decree, i.e. the carbohydrate content was higher than 60 g / 100 g of honey and the sucrose content was lower than 5 g / 100 g of honey.