MICELLAR ELECTROKINETIC CHROMATOGRAPHY FOR IDENTIFICATION OF PHENOLIC COMPOUNDS

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Introduction. Nowadays the interest by search new sources of polyphenols has been increased due to their beneficial properties that have been related to their consumption. Then there is an interest by to develop methods of fast and sensible analyses of these compounds for their identification. One of the current techniques for analysis of antioxidants is the micellar electrokinetic chromatography (MECK) (1, 2) which is a modality of the capillary electrophoresis (CE). In this methodology the addition of surfactants to the buffer produce micelles within the capillary improving the separation, this technique is being a hybrid method between CE and HPLC (3). The MECK methodology is an alternative for the analysis of polyphenols since it diminishes the time and the cost of the analysis. The objective of this work was development a rapid method for identification of phenolic compounds in flour samples *Crataegus pubescens* (Hawthorn) using MECK.

Methods. The experiments to optimize the separation were conducted on a CE system (Beckman-Coulter™ equipment with diode array detector). For all experiments, a fused-silica capillary obtained from Polymicro Technologies (Phoenix, USA) measuring 60 cm x 75 µm internal diameters was used. The used buffer was $Na_2B_4O_7$-KH_2PO_4-SDS. The used standards were: epicatechin, rutin, vitexin-2-ramnoside, isoquercitrin, vitexin, hyperoside, chlorogénic acid and quercetin (degree HPLC). The extracts of the vegetable sample were prepared in ethanol from flour of *Crataegus pubescens* in ethanol.

Results. The optimal separation conditions were found experimentally, the tested factors were: pH, voltage, injection time, injection pressure, and concentration of buffer, the tested ranges of these variables are shown in Table 1. The conditions that had allowed a good resolution of the standards was performed at 24 kV, pH 9, 25 mM buffer with hydrodynamic injection (3 s, 0.5 psi). Under those conditions it was performed the separation of standards in a lower time than 10 minutes (Figure 1). In the same way the coefficient of variation was determined between each analysis (n=6) being smaller than 1%, which indicated a good repeatability of the method.

Table 1. Variables tested in the separation.

Injection time	3-5 s	pH	8.5-9.5
Injection pressure	0.5-1 psi	Voltage	20-30 kV
Buffer Concentration		20-50 mM	

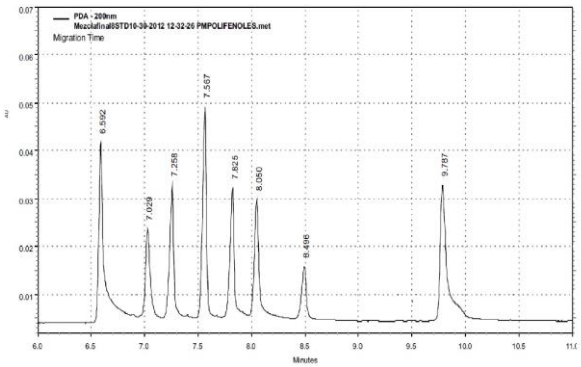

Figure 1. Electropherogram of standards of polyphenols.

In the extract of hawthorn fruit were detected 20 phenolics compounds of which were identified some of them as: chlorogenic acid, rutin, epicatechin; these were confirmed by corresponding standard spectrum.

Conclusions. A faster and repeatable method was developed (smaller than 10 minutes) for identification of polyphenols by MECK. These identified antioxidant in an hawthorn flour can give an added value to the fruit.

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