Application of a new capillary electrophoretic method for the determination of carbohydrates in forensic, pharmaceutical, and beverage samples

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ABSTRACT

A new capillary electrophoresis method dedicated to the analysis of neutral underivatized carbohydrates was recently developed by our group. It involved a background electrolyte composed of 98 mM NaOH and 120 mM NaCl, and direct UV detection via the formation of an absorbing intermediate in the detection window by photooxidation. This article focuses on the validation of this method for the determination of fructose, glucose, lactose, and sucrose in forensic, pharmaceutical, and beverage samples. Intermediate precisions were about 2.3% for normalized corrected peak areas and 1.8% for normalized migration times using naphthalenesulfonate as internal standard. Limits of detection varying from 5 μM for sucrose and lactose to 7 μM for glucose and 10 μM for fructose were obtained. Potential matrix effects were statistically studied for soil, cloth, plastic, cotton, red wine, and with simulated iron, calcium, and sucrose-based matrices, containing various inorganic anions and cations, sometimes at high levels. No significant matrix effect was observed. Finally, analyses of real post-explosion residues, smoke device, cough syrup, red wine, and apple juice were successfully performed.

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1. Introduction

Carbohydrates such as fructose, glucose, lactose, and sucrose are widely distributed in various food, beverage, drug, and forensic samples. Their quantitative analyses allow the evaluation of sample authenticity, the quality of the food products or, for example, provide information on the nature and the composition of carbohydrate-based explosive charges. The most widely employed techniques for carbohydrate analysis is HPLC using normal [1] or reversed phase [2] or anion-exchange modes [2,3] with direct low-UV-range or refractive index detection [4] and most often mass spectrometry or fluorescence detection after precolumn derivatization [5–7]. Important developments involving high performance anion-exchange chromatography coupled to pulsed-amperometric detection were also carried out [3,4].

In recent years, capillary electrophoresis (CE) has been established as a powerful technique for carbohydrates analysis [4,7–11] in several matrices such as fruit juices [12–17], alcohol [14,18,19], dairy products [17,20,21], plant fiber [22] or rat brain [23]. As most carbohydrates do not contain easily ionizable (pKa~12) and

chromophoric groups, many strategies have been developed in order to use the high separation efficiency and speed of CE for carbohydrate analyses such as complexation with borate anions [24,25], precolumn derivatization with a fluorophore [26–29] or the use of an anionic chromophore in alkaline conditions [15,19,30,31] or ionic liquid [32] for their indirect UV-detection. In order to avoid time-consuming and expensive derivatization steps for carbohydrate labeling or the indirect UV detection mode with high alkaline electrolytes, which is poor in sensitivity, a simple method inspired by a former work by Rovio et al. [14,22] has been developed and optimized by our group [33,34]. A separation of 9 carbohydrates involving a percolation of hexadimethrine bromide (HDMB) before each run to reverse EOF, associated with a background electrolyte (BGE) composed of 98 mM NaOH and 120 mM NaCl was optimized with a response surface strategy and a desirability analysis [34]. The direct UV detection of carbohydrates was based on the formation of an absorbing intermediate (malonaldehyde or related compounds) undergoing the low-UV radiations of a Beckman Coulter diode array detector (DAD) [33]. This article focuses on the quantitative validation of the developed method. A chemometric approach has next been involved to study the potential matrix effect with several real extracts of cloth, plastic, soil, wine, and cotton, and of simulated matrices containing high levels of sucrose, iron(II) or calcium ions. Finally, applications to forensic, pharmaceutical, and beverage real samples were carried out.

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2. Materials and methods

2.1. Standards and electrolytes

All high purity carbohydrates used as standard samples (fructose, glucose, lactose, and sucrose) were purchased from VWR (Fontenay-sous-Bois, France). HDMB used as electroosmotic flow reversal agent and naphthalenesulfonic acid (NSA) used as internal standard were purchased from Sigma-Aldrich (L'Isle-d'Abeau, France). 100 mM individual carbohydrate solutions were prepared weekly by volumetric dissolution in ultra-pure water delivered by a Direct-Q3 UV system (Millipore, Molsheim, France). A standard mixture of the carbohydrates of interest was prepared daily (0.05 mM each in ultra-pure water). BGE was composed of 98 mM NaOH and 120 mM NaCl (calculated pH, 13.0). HDMB solution at $1\,g\,L^{-1}$ was prepared by dissolving the appropriate amount in ultra-pure water.

2.2. Sample preparation for forensic, pharmaceutical, and beverage samples

Real samples of red wine (vine plant Merlot from France), apple juice (Pressade, France), and cough syrup (Clarix, COOPER, France) were commercial products and were just diluted with ultra-pure water before analyses. Real forensic samples (smoke device powder and post-blast residues) and blank matrices were collected either directly or via cotton swabs. The first step of this last procedure consisted of the purification of usual hydrophilic cotton swabs by assisted solvent extraction using an ASE 200 instrument (Dionex, Voisin-Le-Bretonneux, France), with one cycle of 5 min at 100 °C and 100 bar with water and next with acetone. Cotton swabs moistened with water were next wiped over samples and were used for inorganic analysis. Post-blast residues, cotton swabs, 1 g of smoke device powder and blank samples were next extracted in boiling water placed in a sonication bath for 10 min. The obtained solutions were filtered through a 150-μm cellulose filter (Les Filtres Durieux, Marne-la-Vallée, France) then through a 0.45-μm nylon syringe filter (Teknokroma, A.I.T France, Houilles, France) and finally diluted just before the injection.

2.3. Apparatus

The CE experiments were carried out with a Beckman Coulter P/ACE MDQ system (Villepinte, France) equipped with a DAD set at 270 nm (analysis wavelength) and 350 nm (reference wavelength) and with bandwidths set at ± 6 nm and ± 40 nm, respectively. Instrument control and data acquisition were performed using Beckman 32 Karat® software.

2.4. Electrophoretic procedures

Electrophoretic separations were performed using 50 μm id × 60 cm bare fused-silica Polymicro capillaries purchased from Photonlines (Marly-Le-Roi, France). A detection window was created for the UV detection at 10 cm from the anodic end. Before first use, capillaries were conditioned by successive flushing with 1 M NaOH, 0.1 M NaOH, ultra-pure water, HDMB solution, and finally BGE, each under 2.8 bar for 3 min (12 capillary volumes), except for HDMB which were under 1.4 bar for 6 min (12 capillary volumes) in order to obtain a better coating and more reproducible separations. Between each run, HDMB layers were refreshed (1.4 bar for 6 min), and this was followed by the percolation of BGE (2.8 bar for 3 min). Injections were performed hydrodynamically under 50 mbar for 5 s (0.75% of the capillary volume). Separations were run at 26.5 °C under -14 kV. BGE was changed between each run.

2.5. Chemometric approach of matrix effect

Chemometric studies of matrix effect were carried out with five repeated injections for five different carbohydrate concentrations (between 15 and 300 μM for sucrose and lactose, and 30 and 600 μM for fructose and glucose) in ultra-pure water for the standard calibration and three repeated injections for the same five carbohydrate concentrations in given matrix extracts (cloth, plastic, soil, wine, and cotton) and in simulated matrices containing high level of sucrose, iron(II) or calcium ions. Extracts of cloth, plastic, soil, and cotton used for matrix effect study were obtained after extraction of blank matrices in boiling water under sonication. Simulated matrices were prepared by dissolution of sucrose, $FeSO_4$, and $CaSO_4$ in water. A wine sample (vine plant Merlot from France) was also selected for the matrix effect study. When matrices already contained some carbohydrates, additional spiking was performed. For simulated sucrose-based matrix, which contained a high content of sucrose, the matrix effect was studied only on fructose, glucose, and lactose. Statistical parameters of the regression lines were computed with Excel® software (Microsoft).

3. Results and discussion

3.1. Quantitative validation

The validation of the previously optimized method [34], enabling the separation of a standard mixture of 9 carbohydrates in 19 min with a 98 mM NaOH and 120 mM NaCl-based BGE, was carried out to evaluate its potential for routine determination of carbohydrates in real extracts. This validation was just performed for the 4 carbohydrates of interest for the Central Laboratory of the Prefecture de Police (fructose, glucose, lactose, and sucrose) according to the ISO/CEI 17025 norm specifications. Naphthalenesulfonate anion was used as internal standard. For the Central Laboratory of the Prefecture de Police de Paris, precision and accuracy of the method had to be better than 5% and 20%, respectively.

3.1.1. Selectivity

The electrophoretic method, optimized using a multivariate approach, led to a complete separation of nine carbohydrates, the four of prime interest plus five other carbohydrates which may be present in natural samples [34]. The most critical pair, galactose/maltose, was the only one presenting a partial resolution, but this did not impact the detection of the four carbohydrates of interest. This lends support to considering that the selectivity of the CE method was satisfactory.

3.1.2. Precision

The precision of the method was evaluated for a mixture of the four carbohydrates at a concentration of 0.05 mM each and 0.34 mM NSA, making five repeated analyses on three different days. Standard mixtures and electrolytes were prepared daily. The responses measured on each electropherogram were the normalized corrected peak areas $((A_i/t_{M,i})/(A_{NSA}/t_{M,NSA}))$ and the normalized migration time $(t_{M,i}/t_{M,NSA})$ of each carbohydrate where A_i stands for carbohydrate peak area, $t_{M,i}$, carbohydrate migration time, A_{NSA}, NSA peak area and $t_{M,NSA}$, NSA migration time. Method repeatability was characterized by the intra-day RSD and method intermediate precision was characterized by the intermediate precision RSD. One way analysis of variance (ANOVA) [35] was used to determine the intra-day (Eq. (1)) and