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Analytical Methods

Determination of alditols by capillary electrophoresis with indirect laser-induced fluorescence detection

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ABSTRACT

A novel method was developed based on capillary electrophoresis with indirect laser-induced fluorescence detection for the determination of alditols including xylitol, sorbitol, arabinitol and mannitol. The baseline separation was achieved with a capillary with 30 μm inner diameter and running buffer (pH 9.6) composed of 40 mM sodium tetraborate, 5% (v/v) methanol, 10^{-5} M sodium fluorescein as background fluorescence. Calibration curves (0.2–2.0 mg mL^{-1}) showed good linear correlations ($r > 0.999$). The detection limits of the alditols were in the range of 19.0–24.4 μg mL^{-1}. The intra-day and inter-day RSDs for migration times and peak areas were less than 1.6% and 4.3%, respectively. The average recoveries were in the range of 99.6–105.5%. The developed method was successfully applied to determination of alditols in different real samples, and the results were compared with those of capillary electrophoresis with indirect ultraviolet detection method.

1. Introduction

Alditols, also known as polyols and sugar alcohols, are the products of hydrogenation reduction of aldose or ketoses. They have been widely used in food and medicine industries due to its outstanding merits such as high thermal stability, low calories and reducing blood sugar levels. Alditols, like xylitol, sorbitol, arabinitol and mannitol, have gained popularity for the replacement of sucrose in confectionery products as high intensity sweeteners without the calories associated with sugars (Sicard, Birch, & Parker, 1982). Besides, sorbitol and mannitol are commonly used as diuretic dehydrants in the treatment of patients with oliguric renal failure (Grunewald & Kinne, 1989; Solomon, Werner, Mann, D'Elia, & Silva, 1994), and D-arabinitol/L-arabinitol ratio in urine and serum of patients can be used as an indicator of clinical diagnosis and monitoring of systemic fungal diseases (Sigmundsdottir et al., 2000; Yeo, Zhang, Schafer, Campbell, & Wong, 2000). Furthermore, with moisture absorption and thermal stability, sorbitol can be also used as moisturizing agent, cryoprotectant and preservative (Chang, Shepherd, Sun, Tang, & Pikal, 2005). Due to the wide applications of alditols mentioned above, the determination of alditols is of great interest to be developed for the quality control of alditols-containing products.

Separation and determination of alditols are difficult because of their unique properties of high pKa values in the range of 12–14, high hydrophily and lack of chromophores. Even so, progress has been made in recent years in the study of analytical methods of alditols which mainly include high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). HPLC which can be coupled with many direct or indirect detections is an efficient method for the determination of alditols (Andersen & Sørensen, 2000; Dona & Verchère, 1995; Kwang-Hyok, Ui-Nam, Sarkar, & Bhadra, 2005; Miyagi, Yokoyama, & Hibi, 2007; Nojiri, Saito, Taguchi, Oishi, & Maki, 1999), however, it usually suffers some drawbacks such as high cost, complicated pre/post-column derivative procedures, a short lifetime of column because of the employment of high pH mobile phase needed to ionise alditols and lack of sensitivity etc. GC method is also commonly used for the determination of alditols, but it requires the conversion of alditols to suitable volatile derivatives (Ratsimba, Fernández, Defaye, Nigay, & Voilley, 1999; Salonen, Rimpilainen, Lehtonen, Lehtonen, & Nikoskelainen, 2001). As an alternative method, CE is a powerful separation technique that can provide high resolution efficiency and require only small amounts of samples and short analysis time. Coupled with direct or indirect ultraviolet detection, CE has been widely applied to separate and analyse carbohydrates by derivatizing the analytes or employing highly alkaline electrolyte (Chen, Jiang,

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Chen, & Qin, 2010; Pospisilova, Polasek, Safra, & Petriska, 2007; Soga & Serwe, 2000; Zemann, Nguyen, & Bonn, 1997). As for direct ultraviolet detection, the required derivatization procedures are not only time-consuming but can also lead to multiple labelling which may reduce peak efficiency and increase the difficulty of separation and detection. As for indirect ultraviolet detection mode, high pH value of electrolyte (pH > 12), which is commonly used for the ionisation of alditols (Soga & Serwe, 2000; Zemann et al., 1997), often causes high *Joule* heat and results in poor peak shape, sensitivity and repeatability. CE coupled with MS (Klampfl & Buchberger, 2001) and electrochemical detection (Chen & Huang, 1997) has also been proposed to analyse carbohydrates, but MS detection are not compatible with most background electrolytes due to their insufficient volatility, and electrochemical detection would be interfered by many factors, thus limiting their wide applications.

One of the most sensitive detection techniques in CE is laser-induced fluorescence detection (LIF) (Wang, Zhou, Wang, Luo, & Hu, 2008), which was first introduced into CE by Gassmann, Kuo, and Zare (1985). However, the general applicability of this technique is limited, as many compounds do not have native fluorescence and fluorescence derivatization would encounter some problems such as scarce fluorescent reagents, instability of some fluorescent reagents and derivatives, lack of derivative specificity and so on. Fortunately, indirect laser-induced fluorescence detection (ILIF) offers an alternative. CE-ILIF method utilises a running buffer containing a fluorescent molecule as probe ion. Due to the requirement of charge neutrality, analytes with like charge will displace the probe and reduce the probe's concentration in the analyte band, and thus produce lowered signals (negative peaks) in the baseline (Yang, Wang, & Zhang, 2006). Although the sensitivity of ILIF is lower than direct LIF detection and almost the same as ultraviolet detection, ILIF is a universal detection method without complicated derivatization. Recently, CE-ILIF method has been extensively used to detect many kinds of compounds (Chen, Sun, Dai, & Min, 2012; Khurana & Santiago, 2008; Szökő & Tábi, 2010; Wang, Tang, Wang, Zhou, & Hu, 2007).

However, to the best of our knowledge, analysis of alditols using CE-ILIF has not been reported. In this work, a new method for the separation and detection of alditols including xylitol, sorbitol, arabinitol and mannitol was developed using CE-ILIF. Fluorescein sodium was selected as the background ion and an all-solid-state laser was used to induce the fluorescence background. Borate buffer was used to produce negatively-charged alditols–borate complexes, resulting in the separation of different alditols by capillary zone electrophoresis (CZE) without the employment of highly alkaline electrolyte (pH > 12). Operation conditions including the pH and concentration of borate buffer, fluorescein sodium concentration, organic modifiers as well as the capillary inner diameter were optimised. The analytical performance of the method was also evaluated. Finally, the developed method was successfully applied to determination of alditols in different alditols-containing products including xylitol mother liquor, xylitol orange juice, xylitol peanut milk and xylitol chewing gum. The results were compared with the data obtained using a method of capillary electrophoresis with indirect ultraviolet detection (CE-IUV).

2. Experimental

2.1. Chemicals and reagents

Arabinitol (>99%) was obtained from Alfa Company (USA). Sorbitol (>98%), mannitol (>98%) and xylitol (>99%) were purchased from Aladdin Company (Shanghai, China). For real samples, xylitol mother liquor was gifted from Futian Pharmacy Co. (Yucheng, Shandong). Tulaotai xylitol orange juice was from Dragon Food Co. (Jiyuan, China). Laobute xylitol peanut milk was from Huiyuan Food & Drink Co. (Beijing, China). Xylitol chewing gum was from Lotte Food Co. (Shanghai, China). Fluorescein sodium was purchased from Kermel Chemical Reagent Development Center (Tianjin, China), which has native fluorescence with the maximum excitation and emission wavelength close to 487 nm and 518 nm, respectively. Sodium tetraborate (99.5%) was purchased from Bodi Chemical (Tianjin, China). All other reagents were of analytical grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Solution preparation

The solution of CE running buffer was freshly prepared as borate buffer (20–50 mM sodium tetraborate at pH 9.0–10.0) with or without organic modifier, containing a certain amount of sodium fluorescein. Stock solutions of alditols (xylitol, sorbitol, mannitol and arabinitol) were prepared in water to give a concentration of 10 mg/mL each. By mixing and diluting the stock solutions with water, the standard mixture solutions of series concentrations were prepared. Three capsules of xylitol chewing gum were finely grinded in a mortar after being frozen, and then precisely weighed powder (0.2 g) was extracted with 2 mL water by sonication for 45 min in total. The obtained solution, as well as xylitol mother liquor, xylitol orange juice and xylitol peanut milk were filtered, respectively, through 0.22 μm cellulose membrane filter (Xinya Purifying Equipments, Shanghai, China). Considering high concentration of alditols, the real samples were all diluted to appropriate concentration with the aqueous solution of internal standard (mannitol) prior to analysis. All other solutions were also filtered through 0.22 μm cellulose membrane filter before use.

2.3. CE-ILIF analysis

We adopted a homemade CE-ILIF instrument with high sensitivity and stability which was described in detail before (Deng et al., 2011, 2014). An all-solid-state laser (10 mW) was utilised as excitation source (473 nm) and the fluorescence emission wavelength was set at 520 nm. High voltage power (±30 kV, 0.3 mA) used was from Shanghai Institute of Applied Physics (China). Data acquisition was performed using N2000 SP1 software (Saizhi Tech., Hangzhou, China). The separation was carried out on a 60 cm (45 cm from inlet to the detector) × 30 μm I.D. (O.D. 365 μm) capillary (Polymicro Technologies, USA). The applied voltage was kept at 20 kV (333 V/cm), resulting in an electrophoretic current of about 0.03 μA, and the capillary temperature was thermostatted at (25 ± 2) °C. An on-column detection window was created by burning off a 5-mm section of the capillary polyimide coating.

Before use, a new capillary was sequentially pre-treated with 1 M hydrochloric acid, water, 1 M sodium hydroxide and water for 30 min each, and then rinsed with the running buffer for 30 min. Before the first run everyday, the capillary was conditioned with 0.1 M sodium hydroxide for 20 min, and then balanced with the running buffer for 15 min. From run to run, the capillary was rinsed with the running buffer for 2 min. Hydrodynamic injection was carried out with sample vial 10 cm higher for 15 s.

2.4. CE-ILIF method validation

The developed CE-ILIF method was applied to alditols determination experiments. Mannitol (1.0 mg mL^{-1}) was used as an internal standard. The linearity was confirmed by five-point calibration curves (0.2, 0.5, 1.0, 1.5, 2.0 mg mL^{-1}) for xylitol, sorbitol and