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Simultaneous separation and determination of erythritol, xylitol, sorbitol, mannitol, maltitol, fructose, glucose, sucrose and maltose in food products by high performance liquid chromatography coupled to charged aerosol detector

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ABSTRACT

A simple, sensitive and accurate method for simultaneous determination of glucose, fructose, sucrose, maltose, erythritol, mannitol, maltitol, sorbitol and xylitol by high pressure liquid chromatography coupled to Corona charged aerosol detector (CAD) is reported for the first time. The method was elaborated using a Shodex Asahipak, NH2P-50 4E, column packed with 5 μm shell particles (4.6 × 250 mm) and acetonitrile–water gradient mobile phase at 25 °C. The method was validated for linearity, precision and accuracy. It was characterized by wide concentration range (1–100 μg ml^{-1}) and good accuracy (95.6–105%). LOD and LOQ for nine analytes were in the range of 0.12–0.44 μg ml^{-1} and 0.40–1.47 μg ml^{-1}, respectively. The method offers excellent linearity with regression coefficient $r > 0.999$ and good repeatability (RSD < 5%). The results obtained for real samples illustrate the ability of the proposed method to quantify a range of sugars and sugar alcohols in a single analysis, making it appropriate for food analysis.

1. Introduction

The Corona CAD is a new type of detector introduced for HPLC applications and has recently become widely used [1–8]. It was introduced by Dixon and Peterson in 2002 [9]. In charged aeorosol detection, column eluent is first nebulized with nitrogen and dried, producing analyte particles. A secondary stream of nitrogen, which becomes positively charged as it passes a high-voltage corona wire, transfers charge diffusionally to the opposing stream of analyte particles. The amount of charge acquired is directly proportional to particle size. It is measured by a highly sensitive electrometer, generating a signal in direct proportion to the quantity of analyte present [10].

There are several application notes of Corona CAD including those for sugar analysis [11,12] but they concern only carbohydrates when there is a need for combined analysis of different nutritive sweeteners such as sugars and polyols.

Sugars and sugar alcohols (polyols) are important part of a human diet. Altogether they are called nutritive sweeteners. Sucrose, glucose, fructose, lactose and maltose are sugars in the diet that originate both from natural sources such as fruits and honey, or from added sugars, mainly sucrose or high-fructose syrup. Sugar alcohols might be added to foods as alternative sweeteners, or be their natural component, and often the foods with these sweeteners are called sugar free. Even though they have no ADI specified, they are known for their potent laxative effect when eaten in excess [13]. At present, EU legislation defines seven sugar alcohols as nutritive sweeteners, i.e. sorbitol (E420), mannitol (E421), isomalt (E953), maltitol (E965), lactitol (E966), xylitol (E967) and erythritol (E968) [14].

Sugars, especially those added at the table or during the processing or preparation of foods, are a major contributor of calories to diet. Increased consumption of added sugars has led to calls for renewed public nutrition efforts including more detailed nutrition labels. Nutrition knowledge and availability of information can affect current and future dietary choices. The nutrition labels clearly identify the number of grams of carbohydrates, however, they do not identify particular sugars. With respect to consumer health it is extremely important to control the amount of sweeteners in food products.

Various analytical methods have been reported for the determination of sugars and sugar alcohols, including gas chromatography [15,16], the high performance liquid chromatography (HPLC) [1,16–19] and capillary electrophoresis (CE) [20]. Gas chromatography requires a time-consuming and laborious sample derivatization to trimethylsilanes or alditol acetates. HPLC methods are better choice as they need only simple water dilutions; however, carbohydrates pose many analytical problems. The lack of any chromophore in sugars and sugar alcohols makes the specific UV detection unreliable or even impossible. Therefore, other universal detectors are used in liquid chromatography to

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detect these compounds, i.e. refractive index (RI) and evaporative light scattering (ELSD). However, RI detection has significant limitations in sensitivity and reproducibility as well as it cannot be used in gradient elution analysis. The ELSD is claimed to be less sensitive and not very precise in comparison with the Corona CAD [2–4]. Although fluorescent detection and mass spectrometry can also be used, they require a lengthy and time-consuming derivatization process as well as are quite expensive. However, sugars and polyols are rarely analyzed simultaneously [19,21].

Therefore, there is a need for elaboration of fast and simple analytical methods which can be easily applied in food analysis for sugars and sugar alcohols determination. Application of the Corona CAD detector that measures a physical property of analyte and responds to almost all non-volatile species, independently of their nature and spectral or physico-chemical properties without any derivatization, could solve all the shortcomings.

The aim of the study was to develop and validate a new chromatographic method for HPLC-CAD, enabling the simultaneous determination of four sugars (glucose, fructose, sucrose, maltose) and five sugar alcohols (erythritol, mannitol, maltitol, sorbitol, xylitol). The procedure was applied to the determination of these nine compounds in various beverages and dietary supplements with very good results.

2. Experimental

2.1. Chemicals, reagents and standards

HPLC-grade acetonitrile was obtained from J.T. Baker (Malinckrodt Baker B.V., Holland) and analytical grade xylitol, D-mannitol, sucrose, D-fructose, D-sorbitol, D-glucose, maltitol, meso-erythritol and D-maltose (all contents > 99%) were purchased from Sigma Aldrich. All aqueous solutions were prepared using ultra pure water (18.2 MΩ cm^{-1}) from a Milli-Q system (Millipore, MA, USA). Standards were made by dissolving 0.10 g of each individual analyte in ultra pure water in separate 100.00 ml volumetric flasks. The standard working solutions (1–100 μg ml^{-1}) were daily prepared by appropriate dilution from the individual stocks. The stock solutions and diluted standard solutions were stored in glass volumetric flasks in the dark at 4 °C.

2.2. Sample preparation

The products analyzed in this study including fruit juices, nectars, fruit beverages and syrups (diet supplements), produced by various companies, were randomly purchased from the local market in Gdansk (Poland). They were kept at room temperature until their analysis.

The analyzed samples were first filtered through filter paper to remove any solid particles and then diluted with 75% acetonitrile as recommended by column manufacturer [22]. All the final solutions were filtered through 0.45 μm membrane filters before injection.

2.3. Instrumentation

Chromatographic experiments were performed on HPLC Ultimate 3000 system (Dionex, Germering, Germany) coupled with a Corona CAD instrument (ESA, Chelmsford, MA, USA). Data processing was carried out with Chromeleon 6.8 software (Dionex) and nitrogen gas flow rate was regulated automatically and monitored by the CAD device. Gas was supplied by nitrogen generator Sirocco-5 (Schmidlin-DBS, Switzerland). The nitrogen gas pressure of the CAD was adjusted to 35 psi, the range was set to 20 pA full scale and medium filter was applied.

2.4. Chromatographic conditions

Chromatographic separation was achieved using Shodex Asahipak, NH2P-50 4E 5 μm (4.6 × 250 mm) column with a mobile phase composed of water (A) and acetonitrile (B) in a gradient run from 90% B to 77% in 22 min and kept constant until 40 min and then column was equilibrated for 5 min. Solvent gradient elution program is presented in detail in Table 1. Each run was completed within 45 min including 5 min equilibration of the column. The column temperature was maintained at 25 °C as well as the autosampler. The injection volume was 10 μl.

2.5. Statistics

Statistical analyses, i.e. linear regression and tests for intercept, slope, intra- and inter-day repeatability were performed by the Statistica for Windows (version 10, Statsoft, 1984–2011, Cracow, Poland) software package.

3. Results and discussion

We proposed a new method to separate glucose, fructose, sucrose, maltose, erythritol, mannitol, maltitol, sorbitol and xylitol in food samples in a single run at room temperature and minimal pretreatment.

3.1. Optimization of HPLC-CAD conditions

The optimum HPLC-CAD conditions were chosen in terms of peak shape, chromatographic analysis time and resolution.

The resolution of investigated compounds was tested by changing the ratio of acetonitrile and water both in isocratic and gradient run. First, some isocratic conditions were examined using flow rates from 0.6 to 1 ml min^{-1} and 30/70, 25/75, 20/80, 18/82 and 15/85 water–acetonitrile mobile phase. Acetonitrile was chosen as it was recommended for carbohydrates analysis as well as for Corona CAD [22,23]. However, it was impossible to obtain satisfactory separation in isocratic run, especially in case of such compounds as sorbitol, mannitol, maltitol and maltose. Results indicated that improving the percentage of water in mobile phase would shorten the retention times, but resolution would decrease.

Therefore, to improve the separation and reduce the time required for analysis, different experiments were carried out in the gradient mode. First, the increasing acetonitrile concentration approach (from 70% to 90 %) was applied but it resulted only in worse separation. Thus, the reversed approach was taken and gradient from 90% to 75% was adopted. Different isocratic flow rates and column temperatures were also checked. Decreasing flow rate from 1.0 to 0.6 ml min^{-1} resulted in time analysis amounting to 70 min. Therefore, gradient flow rate mode was chosen. Such flow programming elution is suitable for the separation of a mixture of homologous compounds including carbohydrates and alcohols [24].

The optimum composition of the mobile phase was selected as gradient of water (A) to acetonitrile (B) (v/v): 90% B to 77% in 22 min and kept constant until 40 min and then column was equilibrated for 5 min (Table 1). The chosen program offered the best resolution of the peaks. The HPLC was operated in a gradient flow mode which allowed on reduction of separation time to 40 min without the loss of detection sensitivity.

Column temperature was also considered as an important factor for separation. Therefore, we tested the column temperature was set to 25,

Table 1
Solvent gradient elution program required for the elution of sugars and polyols.

Time (min)	Acetonitrile percent (%)	Flow (ml min^{-1})
0.00	90	1.40
8.00	84	1.00
20.00	79	0.60
22.00	77	0.60
40.00	77	0.60
43.00	90	1.40
45.00	90	1.40