



Evaluation of a hydrophilic interaction liquid chromatography design space for sugars and sugar alcohols



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ABSTRACT

Based on a column-screening exercise, a column ranking system was developed for sample mixtures containing any combination of 26 sugar and sugar alcohol analytes using 16 polar stationary phases in the HILIC mode with acetonitrile/water or acetone/water mobile phases. Each analyte was evaluated on the HILIC columns with gradient elution and the subsequent chromatography data was compiled into a statistical software package where any subset of the analytes can be selected and the columns are then ranked by the greatest separation. Since these analytes lack chromophores, aerosol-based detectors, including an evaporative light scattering detector (ELSD) and a charged aerosol detector (CAD) were employed for qualitative and quantitative detection. Example qualitative applications are provided to illustrate the practicality and efficiency of this HILIC column ranking. Furthermore, the design-space approach was used as a starting point for a quantitative method for the trace analysis of glucose in trehalose samples in a complex matrix. Knowledge gained from evaluating the design-space led to rapid development of a capable method as demonstrated through validation of the following parameters: specificity, accuracy, precision, linearity, limit of quantitation, limit of detection, and range.

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1. Introduction

Sugars and sugar alcohols can be found naturally in plant tissues and the fiber of fruits and vegetables and are frequently used by the food industry as thickeners or sweeteners as well as by the pharmaceutical industry as inactive ingredients in drug products. Analysis of these compounds is challenging due to their polar nature and lack of a suitable chromophore, rendering the commonly employed reversed-phase (RP) chromatography with ultraviolet (UV) or fluorescence detection impractical without some form of sample derivatization. Chromatographic techniques such as capillary electrophoresis (CE), gas chromatography (GC), and high performance liquid chromatography (HPLC) have been used in the analysis of sugars and sugar alcohols [1–6]. One popular approach for carbohydrate analysis is HPLC using anion-exchange chromatography with pulsed amperometric detection, which has been applied to various matrices [7–12]. Refractive index detectors (RID) have also been used for the direct detection of sugars and sugar alcohols [13,14]; however, RID is often plagued by poor sensitivity and incompatibility with gradient elution. In contrast, applying HPLC in the HILIC

mode with aerosol-based detectors is one of the better options for the separation and determination of sugars and sugar alcohols to achieve retention and selectivity as well as accurate and low level direct detection. The HILIC concept was first applied to the HPLC retention of sugars using polar stationary phases [15,16] although the HILIC terminology was later coined by Andrew Alpert for the separation of proteins, nucleic acids and polar molecules in 1990 [17]. Aerosol-based detectors, such as the evaporative light scattering detector (ELSD), charged aerosol detector (CAD) and nano quantity analyte detector (NQAD), have a broad application base and the concept and operation have been previously described [18–20]. A comparison of the three types of aerosol detectors has also recently been reported [21]. Several researchers have used HPLC-ELSD or HPLC-CAD for the analysis of a few sugars and sugar alcohol mixtures in various matrices [22,23]. In this report, we investigated 16 polar stationary phases and mobile phase combinations in the HILIC mode for the retention and separation of 26 sugar and sugar alcohol analytes. Because all of the analytes tested here lack a sufficient chromophore, aerosol-based detectors were used for the different analyses. A straightforward ranking algorithm was then developed to facilitate simple screening of the stationary phase/mobile phase combinations and identifying those that give the best separation for any given subset of analytes. The algorithm greatly speeds development of an analytical method for such

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compounds by generating the best stationary phase and mobile phase combination for any combination of analytes making method development more efficient. The value of the ranking algorithm is demonstrated here for both qualitative and quantitative applications.

2. Materials and methods

2.1. Chemicals

Acetonitrile and acetone were purchased from EMD Sciences Inc. (Gibbstown, NJ). Deionized water and nitrogen were from an in-house system. The analytes (i.e., sugars, sugar alcohols, and artificial sweetener) were obtained from the vendors listed in Table 1.

2.2. Equipment – design space investigation

The HPLC system consisted of an Agilent 1100 pump and auto sampler (Santa Clara, CA). Detection was performed with an Alltech 3300 ELSD from Buchi Corporation (New Castle, DE) or an ESA Corona CAD from Thermo Scientific (Waltham, MA). The operating conditions for the ELSD were a temperature of 50 °C, nitrogen gas flow of 1.4 L/min and gain setting of 2. The operating conditions for the CAD were a nebulizer temperature of 30 °C, the range set to 200 pA and the nitrogen pressure set to 35 psi. The HPLC columns evaluated are listed in Table 2.

The column temperature was maintained at 25 °C throughout the analysis. The injection volume was 10 µL while the mobile phase flow rate was 1.0 mL/min. For the design space investigation, each analyte was tested on each column using a gradient starting at 10% water and 90% organic (either acetonitrile or acetone) and then a linear gradient to 60% organic in 30 min, followed by a 4 min hold and a return to the starting condition in 0.5 min and then equilibration for 5.5 min for a total run time of 40 min. Single replicates were used to obtain retention data.

2.3. Sample preparation for design space investigation

An individual stock solution of each sugar and sugar alcohol was prepared at 5 mg/mL in water. With the exception of lactitol, raffinose, and maltotriose, samples for HPLC analysis were prepared from the stock by transferring 100 µL of the stock solution into an HPLC vial and then adding 900 µL of either acetonitrile or acetone to be consistent with the mobile phase organic modifier used during the analysis. Due to the solubility of lactitol, raffinose and maltotriose, 100 µL of the stock solution was added to an HPLC vial followed by 100 µL of water and then 800 µL of the appropriate organic solvent. All of these samples were used to acquire the design space investigation data.

2.4. Trehalose analysis method conditions for validation experiments

Final method conditions for glucose analysis in trehalose employed a 150 × 4.6 mm, 2.6-µm particle size Accucore™ HILIC HPLC column with an ESA CAD. Mobile phase A consisted of Milli-Q water and mobile phase B consisted of acetonitrile (HPLC grade). The diluent was a 60/40 (v/v) mixture of acetonitrile and Milli-Q water. For calibration, five glucose standards were prepared over a range of approximately 0.00072 mg/mL–0.0072 mg/mL in diluent. Trehalose samples (USP grade) were prepared at approximately 40 mg/mL on an anhydrous basis. The glucose samples used were USP grade. For HPLC analysis, the column temperature was set to 55 °C with a flow rate of 1.0 mL/min. The injection volume was 10 µL. Mobile phase A was held at 8% for 6.0 min (92% mobile phase B) during which time glucose eluted, followed by an increase in

mobile phase A to 50% at 6.1 min. Mobile phase A was held at 50% until 10.5 min to quickly elute trehalose from the column. To facilitate re-equilibration, mobile phase B was returned to 8% at 10.6 min and held at 8% until 20.0 min for a total run time of 20.0 min.

3. Results and discussion

3.1. Design space investigation

The design space investigation across 26 different analytes (Table 1) with 16 different chromatography columns (Table 2) and two different mobile phase compositions (using the gradient described in the Materials and methods section) produced the results in Tables 3 and 4. The columns used were selected to represent the broad range of commercially-available HILIC stationary phases available, including zwitterionic, amide, diol, triazole, amino, silica, and hydroxyl ligand. Other columns with stationary phases of the same type may exhibit similar selectivity but specific columns not included in this evaluation should be evaluated to assess similarity. Data presented in Tables 3 and 4 include the retention time of each analyte analyzed on all of the stationary phases with both the acetone/water (Table 3) and acetonitrile/water (Table 4) mobile phases. Based on these results, a unique algorithm was developed in JMP® statistical software (SAS Institute Inc.) to subset the data for the analytes of interest, calculate the intervals between each analyte's retention time for the stationary and mobile phases, and then create a table and graphic highlighting the minimum intervals for each stationary and mobile phase. Note that a similar algorithm could be developed in other software such as Excel. These results enable the scientist to identify method conditions (i.e., combination of stationary phase and mobile phase) that have better separations (based upon both resolution and total run time) relative to the others. At any time, additional columns or additional analytes not described here could be analyzed as described above to expand the design space algorithm.

3.2. Application of the HILIC design space screen and algorithm: qualitative and quantitative applications

The value of the JMP® algorithm is evident in its ability to quickly provide a combination of stationary phase and mobile phase that will separate mixtures of known sugars and/or sugar alcohols. These initial method conditions can be used for qualitative analysis or as starting conditions for quantitative analysis (see sections below for relevant qualitative and quantitative case studies).

3.2.1. Qualitative analysis case study

Erythritol, xylitol, dextrose, sucrose, and trehalose are all used as sweeteners in various beverages including flavored waters, energy drinks and teas. As such, it is valuable to have a method that separates all of those components that can be used in a qualitative or quantitative manner to rapidly identify which components are present in a given sample. A subset of these five sugars and sugar alcohols was created from the complete set of 26 sugars and sugar alcohols dataset and then the JMP® algorithm applied. Based on the HILIC screen described above and the associated JMP® algorithm, the plot in Fig. 1 was created.

Fig. 1 shows the retention time of each analyte across each of the 16 columns with the acetonitrile/water mobile phase system. Note for this scenario, while there are five analytes of interest, there are actually six peaks of interest given that dextrose exists as a mixture of two anomers. For this case, it was desirable to separate the two anomers of dextrose. As a result, some stationary phases in Fig. 1 demonstrate six peaks (indicating resolution of the dextrose anomers) while others demonstrate only five peaks (indicating that the dextrose anomers co-elute under those conditions)

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