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Quantitation of carbohydrate monomers and dimers by liquid chromatography coupled with high-resolution mass spectrometry

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ABSTRACT

As remnants of plant wastes or plant secretions, carbohydrates are widely found in various environmental matrices. Carbohydrate-containing feedstocks represent important carbon sources for engineered bioproduction of commodity compounds. Routine monitoring and quantitation of heterogenous carbohydrate mixtures requires fast, accurate, and precise analytical methods. Here we present two methods to quantify carbohydrates mixtures by coupling hydrophilic interaction liquid chromatography with electrospray ionization high-resolution mass spectrometry. Method 1 was optimized for eleven different carbohydrates: three pentoses (ribose, arabinose, xylose), three hexoses (glucose, fructose, mannose), and five dimers (sucrose, cellobiose, maltose, trehalose, lactose). Method 1 can monitor these carbohydrates simultaneously, except in the case of co-elution of xylose/arabinose and lactose/maltose/cellobiose peaks. Using the same stationary and mobile phases as in Method 1, Method 2 was developed to separate glucose and galactose, which were indistinguishable in Method 1. Both methods have low limits of detection (0.019–0.40 μM) and quantification (0.090–1.3 μM), good precision (2.4–13%) except sucrose (18%), and low mass error (0.0–2.4 ppm). Method 1 was robust at analyzing high ionic strength solutions, but a moderate matrix effect was observed. Finally, we apply Method 1 to track concurrently the extracellular depletion of five carbohydrates (xylose, glucose, fructose, mannose, and maltose) by *Pseudomonas protegens* Pf-5, a biotechnologically-important soil bacterial species.

1. Introduction

Carbohydrates, which serve as important carbon sources for cellular metabolism, are essential feedstocks for engineered bioproduction. In the environment, complex cellulosic materials from plant biomass are broken down by microorganisms into bioavailable carbohydrate monomers [1]. Taking advantage of the energy-rich renewable resource of cellulosic biomass, engineered bioproduction employs microbial cell factories to produce value-added products sustainably [2–4]. Therefore, there is special interest in obtaining accurate and precise quantification of the heterogeneous composition of carbohydrates in environmental samples, during chemical hydrolysis of cellulosic polymers, and in biological reactors during bioproduction of value-added products.

A variety of analytical methods have been reported for quantifying mixtures of carbohydrates despite the challenge of separating and detecting analytes that are isomeric and lack chromophores or fluorophores. Previous studies with chromatographic separations have employed gas chromatography [5], reverse phase high performance liquid chromatography (HPLC) [6,7], ligand exchange chromatography [8], or high-performance anion-exchange chromatography (HPAEC) [9]. Common detection methods, which can be coupled with these chromatographic methods, include UV–Vis, fluorescence, or refractive index (RI) detectors. These chromatographic and detection methods are not desirable for high-throughput sample analysis because they require chemical pre-processing involving either derivatization that makes sample preparation tedious or metal ion additives that complicate analysis [10–12]. Furthermore, RI detectors require isocratic elution, which decreases selectivity and sensitivity, and can have high interference from sample matrices [10,11,13]. Capillary zone electrophoresis, which has been used to obtain high-resolution separation of sugar alcohols and carbohydrates, has improved detection limits compared to HPLC coupled with RI or UV–Vis detectors, but is not preferred for mixture analysis due to common co-elution of compounds and difficulty in detecting sucrose (a common dimer) [10]. To overcome the drawbacks of these detection methods, evaporative light-scattering detection (ELSD) has become a popular alternative method to couple with

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Table 1
Analytical performance[a] of Method 1 and Method 2 in MiliQ water.

Carbohydrate	Retention Time (min)	Theoretical *m/z*	Measured *m/z*	Mass Error[b] (ppm)	LOD[c] (μM)	LOQ[c] (μM)	Precision[d] (%RSD at 2.5 μM)	Precision (%RSD at 10 μM)	LOD Comparison (μM)
Method 1									
Ribose	3.25	149.0455	149.0458	2.0	0.30	0.92	10	10	ND[e]
Xylose	4.68	149.0455	149.0455	0.0	0.12	0.37	10	7.5	0.18 (LC-MS) [12]
Arabinose	4.33	149.0455	149.0457	1.3	0.11	0.32	6.9	7.8	ND[e]
Fructose	6.15	179.0561	179.0563	1.1	0.14	0.37	12	12	0.090 (LC-MS) [12] 0.73 (LC-MS) [23] 0.95 (GC-MS) [23] 340 (LC-ELSD) [11] 3.4 (LC-ELSD) [23]
Mannose	8.46	179.0561	179.0563	1.1	0.15	0.44	14	12	ND[e]
Glucose	9.91	179.0561	179.0563	1.1	0.14	0.42	11	13	0.30 (LC-MS) [12] 0.056 (LC-MS) [23] 3.4 (GC-MS) [23] 170 (LC-ELSD) [11] 5.6 (LC-ELSD) [23]
Sucrose	14.88	341.1089	341.1097	2.4	0.24	0.84	18	18	0.18 (LC-MS) [12] 0.85 (GC-MS) [23] 27 (LC-ELSD) [11] 7.0 (LC-ELSD) [23]
Maltose	15.47	341.1089	341.1094	1.5	0.28	0.76	11	8.1	0.018 (LC-MS) [12] 1.7 (GC-MS) [23] 7.5 (LC-ELSD) [23]
Cellobiose	15.38	341.1089	341.1097	2.3	0.36	1.1	13	14	ND[e]
Trehalose	16.26	341.1089	341.1083	1.8	0.019	0.090	7[f]	9[f]	0.028 (LC-MS) [24] 0.022 (LC-MS) [25] 600 (HPLC-RID) [25]
Lactose	15.46	341.1089	341.1083	1.8	1.2	4.7	7[f]	11[f]	2.63–5.84 [14]
Method 2									
Glucose	13.15	179.0561	179.0561	0.0	0.19	0.61	2.4	2.5	See above
Galactose	11.70	179.0561	179.0561	0.0	0.40	1.3	3.7	3.6	750 (LC-ELSD) [11]

[a] The values in the table were obtained from six replicates (n = 6).
[b] Mass error was calculated in parts-per-million (ppm).
[c] Limit of detection (LOD) and limit of quantification (LOQ).
[d] Relative standard deviation (RSD).
[e] Not Determined.
[f] These values were obtained from five replicates (n = 5).

chromatography for detecting carbohydrates [10,11,14,15]. While ELSD enhances the analyte intensity over RI detectors, the sensitivity can still be poor, and quantification can be challenging due to nonlinear detector responses [10,11]. The combination of HPAEC with pulsed amperometric detection (PAD) has been used as a high-resolution, sensitive, and selective technique to analyze carbohydrates at low concentrations [10]. However, the application of HPAEC-PAD requires highly alkaline conditions and suffers from non-linear detector response and baseline shifting [10,11,16].

Besides chromatography-based methods, both Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy have been used to analyze carbohydrates without prior separation. In FTIR application, which does not require extensive sample preparations or expensive pre-processing reagents, the sensitivity and accuracy are typically poor [10,17]. On the other hand, NMR presents an effective non-derivatization method for monitoring single carbohydrates from mixtures including monosaccharides, disaccharides, and trisaccharides [18,19]. To overcome the poor the sensitivity of NMR, recent studies have reported the use of high fields, cryogenic probes, dynamic nuclear polarization, chemical shift selective filtration, and total correlation spectroscopy [18,19]. Without these enhancing approaches, the sensitivity of NMR-based detection (millimolar to molar concentrations) is several orders of magnitude higher than the sensitivity of mass spectrometry (MS)-based detection (nanomolar to micromolar concentrations) [19]. However, these additional methods can complicate analysis and high ionic strength solutions can still interfere with the sensitivity [19].

Application of MS with electrospray ionization (ESI) has been successful at distinguishing between carbohydrate homologues of different masses and, when coupled with HPLC, can differentiate carbohydrate isomers [12]. Coupling HPLC with ESI-MS can achieve low detection limits, high selectivity, and robust analysis of different carbohydrates in a mixture [6,12,13]. Recently, amide columns perform reproducible and robust separations of carbohydrates based on hydrophilic interactions [12,13,20–22]. At high temperatures and alkaline conditions, amide columns minimize salt interferences, anomer mutarotation, and Schiff base formation [20]. By combining hydrophilic-interaction-based HPLC with MS/MS analysis, sixteen carbohydrates were quantified in a mammalian plasma matrix [12].

In this Research Note, we employ ultra-high performance LC (UHPLC) with an amide column and high-resolution MS (HRMS) with ESI to separate and quantify carbohydrate mixtures relevant to environmental matrices and bioproduction, yielding good sensitivity, selectivity, a large linear dynamic range, and good separation of the majority of analytes. Specifically, we achieve robust and precise quantification of pentoses (xylose, arabinose, and ribose), hexoses (glucose, galactose, mannose, and fructose), and dimers (maltose, cellobiose, sucrose, trehalose, and lactose). These carbohydrates are representative of both reducing and nonreducing sugars that are important for monitoring environmental matrices and for sustainable conversion of biomass to natural products. Although eight of the targeted carbohydrates (xylose, glucose, fructose, galactose, sucrose,

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