Journal of Chromatography A, 1513 (2017) 210–221

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Identification and deconvolution of carbohydrates with gas chromatography-vacuum ultraviolet spectroscopy

Jamie Schenk[a], Gabe Nagy[b], Nicola L.B. Pohl[b], Allegra Leghissa[a], Jonathan Smuts[c], Kevin A. Schug[a,*]

[a] Department of Chemistry & Biochemistry, The University of Texas at Arlington, Arlington, TX 76019, United States
[b] Department of Chemistry, Indiana University, Bloomington, IN 47405, United States
[c] VUV Analytics, Inc., Cedar Park, TX 78613, United States

ARTICLE INFO

Article history:
Received 26 March 2017
Received in revised form 28 June 2017
Accepted 16 July 2017
Available online 18 July 2017

Keywords:
Covariance analysis
Sugars
Saccharides
Cold medicine
Sugar substitutes

ABSTRACT

Methodology for qualitative and quantitative determination of carbohydrates with gas chromatography coupled to vacuum ultraviolet detection (GC-VUV) is presented. Saccharides have been intently studied and are commonly analyzed by gas chromatography-mass spectrometry (GC–MS), but not always effectively. This can be attributed to their high degree of structural complexity: α/β anomers from their axial/equatorial hydroxyl group positioning at the C1-OH and flexible ring structures that lead to the open chain, five-membered ring furanose, and six-membered ring pyranose configurations. This complexity can result in convoluted chromatograms, ambiguous fragmentation patterns and, ultimately, analyte misidentification. In this study, mono-, di, and tri-saccharides were derivatized by two different methods—permethylation and oximation/pertrimethylsilylation—and analyzed by GC-VUV. These two derivatization methods were then compared for their efficiency, ease of use, and robustness. Permethylation proved to be a useful technique for the analysis of ketopentoses and pharmaceuticals soluble in dimethyl sulfoxide (DMSO), while the oximation/pertrimethylsilylation method prevailed as the more promising, overall, derivatization method. VUV spectra have been shown to be distinct and allow for efficient differentiation of isomeric species such as ketopentoses and reducing versus non-reducing sugars. In addition to identification, pharmaceutical samples containing several compounds were derivatized and analyzed for their sugar content with the GC-VUV technique to provide data for qualitative analysis.

1. Introduction

Whereas carbohydrate separation, classification, and identification has received considerable attention in recent years, the analytical toolbox remains bare in large part due to the high degree of structural complexity of this class of analytes [1]. This complexity arises from the number of carbon atoms in the molecule overall and in the individual closed ring (pyranose/furanose) structures, the stereochemistry of the ring substituents including the anomeric configurations (α/β), and the varying functional groups. This incredible diversity of structure in turn translates into a diversity of functions in humans, plants, and the food and pharmaceutical industries [2–5].

Given this diversity, the separation and identification of a wide assortment of carbohydrates, with a specific emphasis on isomeric differentiation, is needed. Nuclear magnetic resonance (NMR) remains the gold standard for structural assignment, but requires milligram scales of sample as a rule and its deconvolution of coupling constants or *J* values from a mixture of saccharides is non-trivial [6]. Traditional liquid chromatography (LC) suffers from column stability, regeneration times, reproducibility of retention times, cost, and the availability of suitable stationary phases [2]. Hydrophilic interaction liquid chromatography, ligand exchange chromatography, and other LC methods have been shown to be inefficient for separation of *all* carbohydrates or complicated isomeric mixtures, even on various stationary phases [7]. High-performance anion-exchange chromatography requires strong concentrations of sodium hydroxide, which makes it challenging to couple to other analytical techniques and/or detectors, and is limited to samples that are resistant to base [8].

Mass spectrometry [1,6] has shown promise for the individual discrimination of hexose and pentose isomers through the mea-

* Corresponding author at: 700 Planetarium Pl., Box 19065, Arlington, TX, 76019-0065, United States.
E-mail address: kschug@uta.edu (K.A. Schug).

http://dx.doi.org/10.1016/j.chroma.2017.07.052
0021-9673/

surement of dissociation rates, but has not been shown to resolve isomeric mixtures. Ion mobility spectrometry-mass spectrometry has been used to discriminate individual glucose isomers [9] but when analyzing isomers, two mobility peaks may not necessararily indicate the presence of two unique isomers, but instead may indicate the presence of two ion conformations. Gas chromatography coupled with mass spectrometry detection suffers similar drawbacks, namely significant coelution and inability to resolve complex mixtures due to similarities in carbohydrate electron ionization mass spectra.

A modern bench-top vacuum ultraviolet detector (VUV) has been introduced that may be able to overcome these shortcomings and be used for the differentiation of mixtures of carbohydrate isomers. Fig. 1 shows a generalized schematic of the GC-VUV instrument [10]. Analytes elute from the GC column and are passed through a heated transfer line into a flow cell. Reflective optics direct light from a deuterium lamp source through the flow cell and to a diffraction grating, which diffracts the light (120–240 nm) for detection by a charge-coupled device (CCD), with a maximum sampling rate of 100 Hz. Perhaps most novel is the fact that the natural additivity of absorbance spectra allows for deconvolution of coeluting peaks [10,11]. These and other merits of the VUV detector have been exemplified previously in the analysis of permanent gases [12], pesticides [13], fatty acids [14,15], terpenes [16], and hydrocarbon fuels [10,11,17,18]. The ability to rapidly deconvolve species in an automated fashion from chromatographic separations of complex mixtures—a process termed time interval deconvolution—has also been demonstrated for PIONA (paraffins, isoparaffins, olefins, naphthenes, and aromatics) analysis of gasolines [19] and polychlorinated biphenyl-containing Aroclor mixtures [20].

The aim of this study was to evaluate the potential of GC-VUV for effective separation, deconvolution, and determination of isobaric and isomeric carbohydrate analytes. Herein, methods are described for the rapid differentiation of mono-, di-, and trisaccharides that have been derivatized by permethylation and oximation/pertrimethylsilylation (O/TMS), so as to become sufficiently volatile and thermally stable for GC analysis. Mixtures of derivatized carbohydrates were analyzed and deconvolutions were performed on compounds that coeluted to separate the compounds for quantitative and qualitative analysis. Covariance calculations were performed to evaluate theoretical detection limits for deconvolutions to compare with experimental measurements. Furthermore, experiments were performed to identify carbohydrates present in samples of over-the-counter cold medicines and prescription medications.

2. Material and methods

2.1. Materials

D-(−)-ribose (99%), *D*-(+)-xylose (99%), *D*-(+)-glucose (99.5%), *D*-(−)-arabinose (98%), *D*-(−)-fructose (99%), *D*-(+)-galactose (99%), *D*-(+)-mannose (99%), sucrose (99.5%), β-lactose (99%), *D*-(+)-cellobiose (98%), melibiose (98%), maltitol (98%), *D*-(+)-turanose (98%), lactulose (98%), sodium hydroxide (98%), iodomethane (99%), pyridine anhydrous (99.8%), hexamethyldisilazane (99%), hydroxylamine hydrochloride (99%), isomaltotriose (98%), *D*-(+)-melezitose hydrate (99%), *D*-(+)-raffinose pentahydrate (98%), 1-kestose (98%), and dimethylsulfoxide (99.5%) were all purchased from Sigma-Aldrich (St. Louis, MO). *D*-ribose, *L*-ribose, *D*-arabinose, *L*-arabinose, *D*-xylose, *L*-xylose, *D*-lyxose, *L*-lyxose, *D*-allose, *L*-allose, *D*-altrose, *L*-altrose, *D*-glucose, *L*-glucose, *D*-mannose, *L*-mannose, *D*-gulose, *L*-gulose, *D*-idose, *L*-idose, *D*-galactose, *L*-galactose, *D*-talose, *L*-talose, *D*-ribulose, *L*-ribulose, *D*-xylulose, *L*-xylulose, *D*-psicose, *L*-psicose, *D*-sorbose, *L*-sorbose, *D*-fructose, *L*-fructose, *D*-tagatose, and *L*-tagatose were obtained from Carbosynth (Berkshire, UK) and Sigma Aldrich (Milwaukee, WI, USA). *L*-ribulose, *D*-xylulose, *L*-idose, and *D*-allose were purchased from Carbosynth (Berkshire, UK). All samples were analyzed at the University of Texas at Arlington (Arlington, TX).

2.2. Instrumentation

A Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) was coupled with a VGA-100 VUV detector (VUV Analytics, Inc., Cedar Park, TX). The GC-VUV set up was used to collect data from derivatized carbohydrate samples at a data acquisition rate of 10 Hz. The transfer and flow cell temperatures were set to 300 °C and nitrogen was used as the makeup gas at 0.25 psi. An Rtx-5 column (30 m × 0.25 mm × 0.25 μm) obtained from Restek Corporation (Bellefonte, PA) was used at a constant velocity of 30 cm/s with a helium carrier gas. The injection port was set to 270 °C with an 8.5:1 split ratio for permethylated samples and 50:1 split for O/TMS samples with a 1.0 μL injection volume.

The GC oven profile for all permethylated monosaccharaides was set to 70 °C for 1 min, followed by a 20 °C/min ramp to 210 °C and held for 8 min, then a 20 °C/min ramp to 300 °C and held for 10 min. The GC oven profile for all O/TMS mono- and disaccharides and permethylated disaccharides was set to 100 °C for 5 min, followed by 10 °C/min ramp to 300 °C and held for 5 min. The GC oven profile for all trisaccharide analysis was set to 200 °C for 15 min, followed by 15 °C/min ramp to 270 °C, then 1 °C/min ramp to 290 °C, then a 15 °C/min ramp to 330 °C and held 15 min. Samples of medicines were analyzed using an oven program initially set at 100 °C for 5 min, followed by a 10 °C/min ramp to 270 °C, followed by a 1 °C/min ramp to 290 °C, followed by a 15 °C/min ramp to 330 °C and held for 15 min. Needle wash solvent was chloroform for permethylated samples and dichloromethane (DCM) for O/TMS samples.

2.3. Permethylation of standards

Each monosaccharide was weighed on a balance (4–5 mg). DMSO (0.5 mL) was added and stirred until the sample was completely dissolved. Powdered NaOH (12 mg) was added, followed immediately by methyl iodide (0.1 mL). The reaction was capped and then stirred for 1 h at ambient temperature. The reaction was diluted with deionized water (2 mL) and chloroform (2 mL). The organic layer was washed three times with deionized water, dried over sodium sulfate, and concentrated. The permethylation method followed previously described methods in the literature [21,22]. The structures of permethylated carbohydrates are shown in the electronic Supplementary Information document, Figs. S1–S3.

2.4. Oximation/pertrimethylsilylation of standards

Monosaccharides were accurately weighed out (5 mg) and dissolved samples (1 mg/mL in methanol:water 30:70, v/v) were concentrated under reduced pressure at 50 °C until dry. Anhydrous pyridine (1.8 mL) was added (containing 2.5 g of hydroxylamine per 100 mL of pyridine) and heated to 70 °C for 30 min. Samples were cooled and hexamethyldisilazane (HMDS) (0.9 mL) and trifluoroacetic acid (TFA) (0.1 mL) were added. Once HMDS and TFA were added, the samples were heated to 100 °C for 60 min. Samples were centrifuged and the supernatant was used for analysis. The oximation/pertrimethylsilylation procedure followed previously described methods in the literature [23–27]. The structures of O/TMS-derivatized carbohydrates are shown in the electronic Supplementary Information document, Figs. S4–S7.