Talanta 159 (2016) 40–46

Contents lists available at ScienceDirect

Talanta

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

New evidences on efficacy of boronic acid-based derivatization method to identify sugars in plant material by gas chromatography–mass spectrometry ☆

Marianna Faraco *, Daniela Fico, Antonio Pennetta, Giuseppe E. De Benedetto

Laboratorio di Spettrometria di Massa Analitica ed Isotopica, Dipartimento di Beni Culturali, Università del Salento, Lecce, Italy

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
31 May 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
Carbohydrate
Gas chromatography–mass spectrometry
Methylboronic acid
Plant material

ABSTRACT

This work presents an analytical procedure based on gas chromatography–mass spectrometry which allows the determination of aldoses (glucose, mannose, galactose, arabinose, xylose, fucose, rhamnose) and chetoses (fructose) in plant material.

One peak for each target carbohydrate was obtained by using an efficient derivatization employing methylboronic acid and acetic anhydride sequentially, whereas the baseline separation of the analytes was accomplished using an ionic liquid capillary column. First, the proposed method was optimized and validated. Successively, it was applied to identify the carbohydrates present in plant material. Finally, the procedure was successfully applied to samples from a XVII century painting, thus highlighting the occurrence of starch glue and fruit tree gum as polysaccharide materials.

1. Introduction

Carbohydrates represent the major form of photosynthetically assimilated carbon in the biosphere. Because of their ubiquity and abundance, saccharides are potentially useful compounds in elucidating sources, processes and pathways of biologically important organic materials in natural environments [1].

Despite their importance, carbohydrates analysis is still a major challenge [2–4]; in particular, the similar molecular weights of the carbohydrate moieties and the large number of isomers make the chromatographic analysis of carbohydrates particularly difficult [4]. Additionally, the presence of different tautomeric forms in solution leads to complex chromatograms [5]. Thanks to its high sensitivity and selectivity, gas chromatography coupled to mass spectrometry (GC–MS) has proven to be the optimal choice for the study of carbohydrates [6]. However, before analysis by GC–MS, all carbohydrates require a suitable derivatization to convert them into volatile derivatives since they naturally exhibit high polarity, pronounced hydrophilicity with a strong tendency to hydrogen bonding, and near-zero volatility [7]. The derivatization strategies shield polar groups and increase volatility (thus improving signal intensity and compound stability), and enhance the quantification and the information content of the mass spectra [8].

Analytical derivatization of carbohydrates has been extensively addressed in the literature, and many derivatization procedures have been developed employing various reagents and reaction conditions [3,4]. In particular, methyl, trifluoroacetyl, trimethylsilyl and tert-butyldimethylsilyl ethers have been largely used as derivatives but a different compound for each anomeric form of sugar [7] is obtained. Several attempts have been made to reduce the number of chromatographic peaks of each derivatized carbohydrate. An approach that is commonly used is to convert the free carbonyl groups into oximes or N-alkyl oximes before silylation: this leads to the presence of only two peaks for each reducing sugar (corresponding to E and Z isomers of the oxime). These derivatives are applicable to both aldoses and ketoses and have been widely used for carbohydrate determinations of complex mixtures [4,9,10]. However, the formation of two derivatives can also be an inconvenience when a complex mixture has to be separated or the concentrations of the analytes are near the detection limits.

Diethyl mercaptal derivatization followed by silylation [11] results in one chromatographic peak for each aldose and uronic acid, and the method could be adapted to analyze polysaccharides present in works of art [5]. More specifically, this method consisted of three steps: a microwave-assisted acidic hydrolysis; a cleanup step to eliminate inorganic material; and, finally, the derivatization using mercaptalation followed by silylation.

A different approach to get only a peak for glucose is the butylboronic derivatization method [12]. In this method, which is commonly used to determine the isotopic composition of 13C-

☆Dedication in memory of Pier Giorgio Zambonin.

* Corresponding author.
E-mail address: marianna.faraco@unisalento.it (M. Faraco).

http://dx.doi.org/10.1016/j.talanta.2016.06.004
0039-9140/

labeled glucose in human plasma by GC-C-IRMS, the butylboronic acid reacts with the alcohol groups of glucose followed by acetylation of the remaining free hydroxyl group. This derivatization reaction is rapid, and this method is less time-consuming as no reduction of the carbonyl group is needed.

In such a context, in the present work, a GC–MS methodology to simultaneously separate, identify and quantify the most common monosaccharides in plant gums and extracts is reported: the proposed methodology is based on methylboronic derivatization followed by acetylation. Optimization of the latter step was necessary to avoid per-acetylation. The effectiveness of this method was validated by assessing the linear range, the limits of detection and quantification, the accuracy, and the repeatability. It was demonstrated that the presence of metal ions was not detrimental to the derivatization efficiency; therefore, the cleanup step to eliminate the inorganic material was not necessary. The monosaccharide profiles for selected plant gums were determined and compared to the data reported in the literature [5]. Finally, the method was used to determine the polysaccharide content in plant material and in samples from a XVII century painting under restoration.

2. Experimental

2.1. Chemicals and reagents

Analytical grade fucose, arabinose, xylose, mannose, rhamnose, fructose, galactose, glucose and mannitol were obtained from Sigma–Aldrich (Milan, Italy). Trifluoroacetic acid (99%) and anhydrous pyridine were purchased from Fluka (Milan, Italy). Methylboronic acid, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), calcium sulphate hemihydrate, cadmium nitrate tetrahydrate, copper(II) chloride, and hexadecane were acquired from Sigma–Aldrich. Acetic anhydride was provided by Supelco Inc. (Bellefonte, PA, USA).

Stock solutions of monosaccharides with concentrations of 1000 μg/mL were prepared in ultrapure water (Integra UV, Diessechem, Italy). The solutions were stored at 4 °C.

2.2. Raw materials

The materials used in this study are detailed in Supplementary Table 1S. *Natural gypsum, French ochre, Raw sienna, Calcium carbonate, dark Naples Yellow, and reddish Naples Yellow* were obtained from Kremer Pigmente (Aichstetten, Germany). The paint on canvas “Sacro Cuore” (Chiesa Parrocchiale, Cursi, Italy) dates back to the XVII century. The sample weight was 0.8 mg.

2.3. Apparatus and chromatographic conditions

A Bruker SCION-456GC gas chromatograph equipped with PAL autosampler and coupled to a Bruker SCION TQ mass spectrometer was used with an SLB IL60 ionic liquid capillary column (60 m × 0.25 mm i.d., 0.25 μm thickness). The injector temperature was 220 °C. Unless otherwise specified, 1.0 μL of the sample was injected in split mode (1:20). Analytes were separated using helium (purity 99.9995%) as carrier gas at a constant flow of 1.2 mL/min with the following oven temperature program: 100 °C (2 min) to 120 °C at 10 °C/min; then to 140 °C (held 5 min) at 2 °C/min; and, finally to 260 °C (held 5 min) at 5 °C/min. The EI spectra were acquired in full scan mode in the range *m/z* 40–500.

2.4. Analytical procedure

Samples (0.1–0.5 mg) were placed in Teflon closed vials, then 10 μL of a 1 mg/mL mannitol solution as derivatization internal standard (IS) and 1 mL of 2 M trifluoroacetic acid were added. The acidic hydrolysis of the samples was then performed using a microwave oven Milestone model ETHOS (Sorisole, Bergamo, Italy) with the following program: power 500 W, temperature 100 °C, duration 30 min. After centrifugation at 10,000 rpm for 5 min in order to remove insoluble matter, supernatant was dried. Each sample was treated first with 100 μL of a 5 mg/mL methylboronic acid in pyridine solution at 100 °C for 10 min; successively, hexadecane (10 μL of a 40 μg/mL solution in isooctane) and 20 μL of acetic anhydride in pyridine (v/v, 1/10) were added at room temperature and, after a reaction time of 60 min, injected. All standards, reference materials and samples were analyzed in triplicate with the optimized method. All quantifications were performed using mannitol as IS.

2.5. Multi-response optimization and data analysis

The experimental design and principal component analysis (PCA) were performed and evaluated using the MINITAB software. A central composite design (CCD) with a quadratic model consisting of 3 factors was applied to optimize the acetylation conditions. CCD is one of the most used techniques in chemometrics for the optimization of chromatographic procedures [13–16]. The mathematical function assumed for each response in terms of the selected independent variables can be expressed by an empirical second-order model Eq. (1).

$$\hat{y}_i = \beta_0 + \sum_{i-1}^{i=k} \beta_i x_i + \sum_{i=1}^{i=k} \sum_{j-1}^{j-k-1} \beta_{ij} x_i x_j + \sum_{i=1}^{i=k} \beta_{ii} x_i^2 \quad \text{(Eq. 1)}$$

In this equation $\hat{y}_i$ represents the predicted response, β_0 is the intercept coefficient (offset term), β_i is the regression coefficient of the linear effect, β_{ii} is the coefficient of the quadratic effect, β_{ij} represents the coefficient of the interaction effect, x_i and x_j are the independent variables (in coded values) and k represents the number of independent variables.

Four monosaccharides (namely, arabinose, xylose, glucose, and galactose), were selected for optimization. The independent variables were temperature, duration and solvent composition. The responses were the peak areas normalized to the peak area of hexadecane (which is not derivatizable) and the number of artifact peaks detected in the chromatograms. The artifact peaks were quantified as the number of spurious peaks between the first and last eluted analytes (hexadecane and galactose). The aim was to obtain the maximum yield of carbohydrate derivatives, and the minimum number of artifact peaks. Each independent variable was evaluated at five different levels for a total of 20 experiments including six replicates at the centre point coded as zero level. All the experiments were carried out randomly, and Supplemental Table 2S shows the matrix of the CCD.

PCA is generally used to reduce the number of observed variables to a smaller number of principal components (PCs) which account for most of the variance of the original data [17,18]. Since the first few components often account for the majority of this multi-dimensional variation, PCA effectively reduces the dimensionality of the data set enabling the differentiation of samples that may otherwise prove difficult to distinguish. A total of 28 reference samples of arabic, tragacanth, fruit-tree and starch glues and the painting sample discussed in the paper were analyzed with the optimized method. Each sample was analyzed in triplicate and the mean values of the relative percentages were used after autoscaling for PCA.

2.6. Method validation

A stock solution containing the mixture of monosaccharides