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Optimized GC-MS Method To Simultaneously Quantify Acetylated Aldose, Ketose, and Alditol for Plant Tissues Based on Derivatization in a Methyl Sulfoxide/1-Methylimidazole System

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Supporting Information

ABSTRACT: The isomers of monosaccharide always produce multiple chromatographic peaks as volatile derivatives during gas chromatography, which may result in the overlapping of different sugar peaks. Whereas reduction and oximation of sugar carbonyl groups for GC analysis do eliminate many isomer derivatives, the approaches create new problems. One ketose can yield two peaks by oximation, and different aldoses and ketoses can yield the same alditol upon reduction, leading to the inability to detect some important monosaccharides. This paper reports an optimal method that yields a single peak per sugar by acetylation directly. By using a methyl sulfoxide (Me_2SO)/1-methylimidazole (1-MeIm) system, the carbohydrates in acetic anhydride (Ac_2O) esterification reactions were solubilized, and the oxidation that normally occurs was inhibited. The results demonstrate that acetylated derivatives of 23 saccharides had unique peaks, which indicates aldose, ketose, and alditol can be determined simultaneously by GC-MS.

KEYWORDS: *aldose, ketose, methyl sulfoxide, acetic anhydride, 1-methylimidazole, GC-MS*

INTRODUCTION

To date, a variety of sugar derivatives for GC are employed. Traditionally, monosaccharides are substituted into silylated derivatives for GC or GC-MS analysis.[1−3] However, there are two problems with silylation.[1,4−6] First, the reagents break down rapidly into unavailable compounds in an aqueous environment. Second, the silylated derivatives exist in anomeric forms: they are readily hydrolyzed by water and thus capable of mutarotation, which leads to multiple peaks on gas chromatography.[4] Because the disadvantages of silylation exist, acetylation is also widely used in GC or GC-MS analysis for saccharides.[7−9]

As early as 1967, Nevins et al. reported that sugars liberated from polysaccharides of plant cell walls could be reduced to their respective alditols, which could then be acetylated and separated by gas chromatography.[10,11] Although the reduction of monosaccharides with sodium borohydride ($NaBH_4$) is highly effective and eliminates the possibility of anomeric derivatives, additional problems can occur during the reaction. Due to the same chemical group and configuration of chiral carbon atoms in the resulting alditol, different aldoses can yield the same alditol upon reduction. For example, D-arabinose and D-lyxose yield the same alditol, D-arabinitol. Things are further complicated when the reduction approach is applied in the derivatization of ketoses. Because of the two possible ways in which OH groups may be attached at C-2, the reduction of a ketose can lead to two alditols that are C-2 epimers[12] (Figure 1E). Besides, the basic borohydride reduction promotes 1,2-enediol formation with reduction capturing the C-2 epimers. For example, glucose in alkaline borohydride always has a proportion of mannitol in addition to the predominant glucitol.[13,14]

Another commonly used approach is to convert the free carbonyl group of an aldose into oxime before acetylation, which in turn creates aldononitrile acetates. Because these derivatives preserve the unique 1-position with the formation of a nonisomeric functional group (nitrile), each aldose gives a single unique peak (Figure 1A). Unfortunately, the approach does not work for ketoses because the oximated, acetylated ketose will yield two peaks due to the *syn* and *anti* isomers[15,16] (Figure 1D).

Prior to acetylation, the aim of oximation or reduction is to eliminate the possible isomer derivatives. However, one ketose

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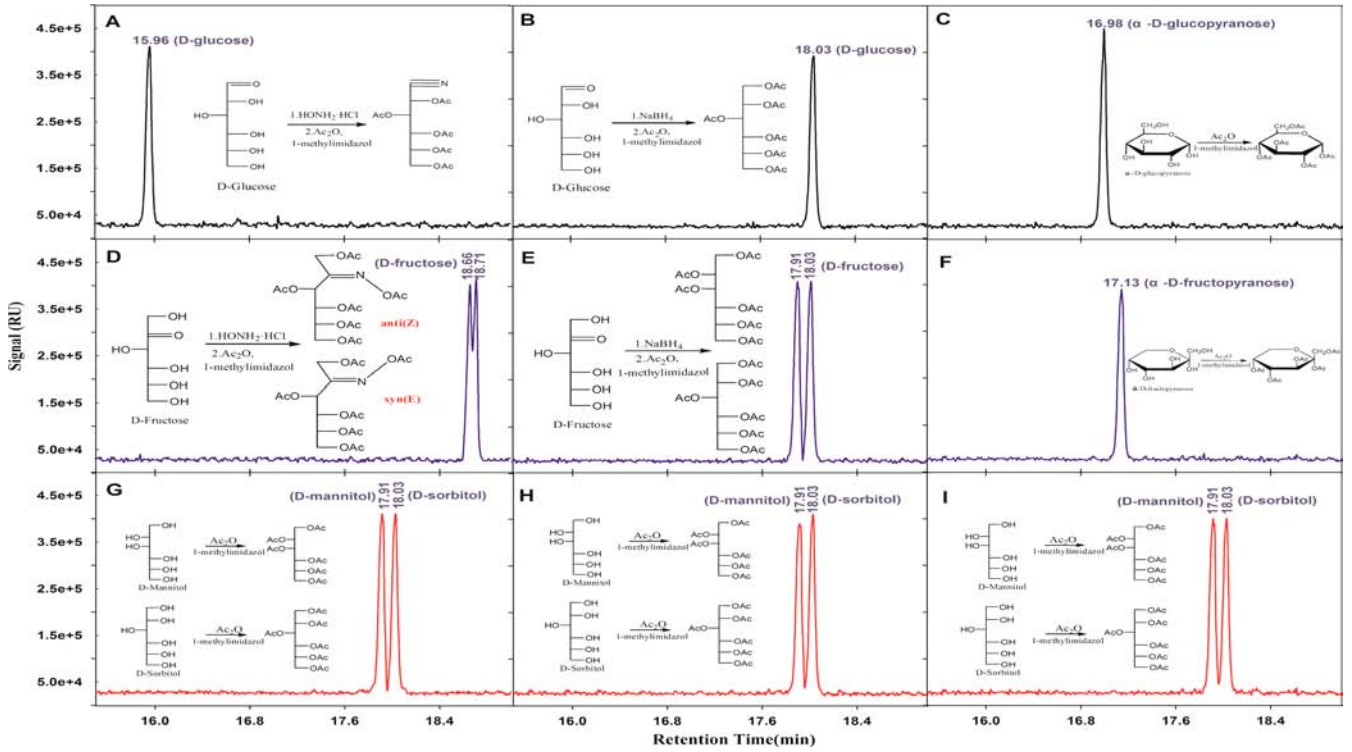

Figure 1. Comparison of three derivatization approaches (oximation and acetylation, reduction and acetylation, acetylation in Me_2SO/1-MeIm) for monosaccharide analysis by GC-MS: (A, D, G) oximation and acetylation [(A) oximated, acetylated D-glucose; (D) oximated, acetylated D-fructose yields two peaks (the *syn* and *anti* isomers) that cannot be separated on a DB-17 MS column; (G) acetylated D-mannitol and D-sorbitol]; (B, E, H) reduction and acetylation [(B) D-glucose is reduced to D-sorbitol; (E) D-fructose is reduced to D-mannitol and D-sorbitol; (H) acetylated D-mannitol and D-sorbitol]; (C, F, I) acetylation in Me_2SO/1-MeIm [(C) locked-ring acetylated α-D-glucopyranose; (F) locked-ring acetylated α-D-fructopyranose; (I) acetylated D-mannitol and D-sorbitol].

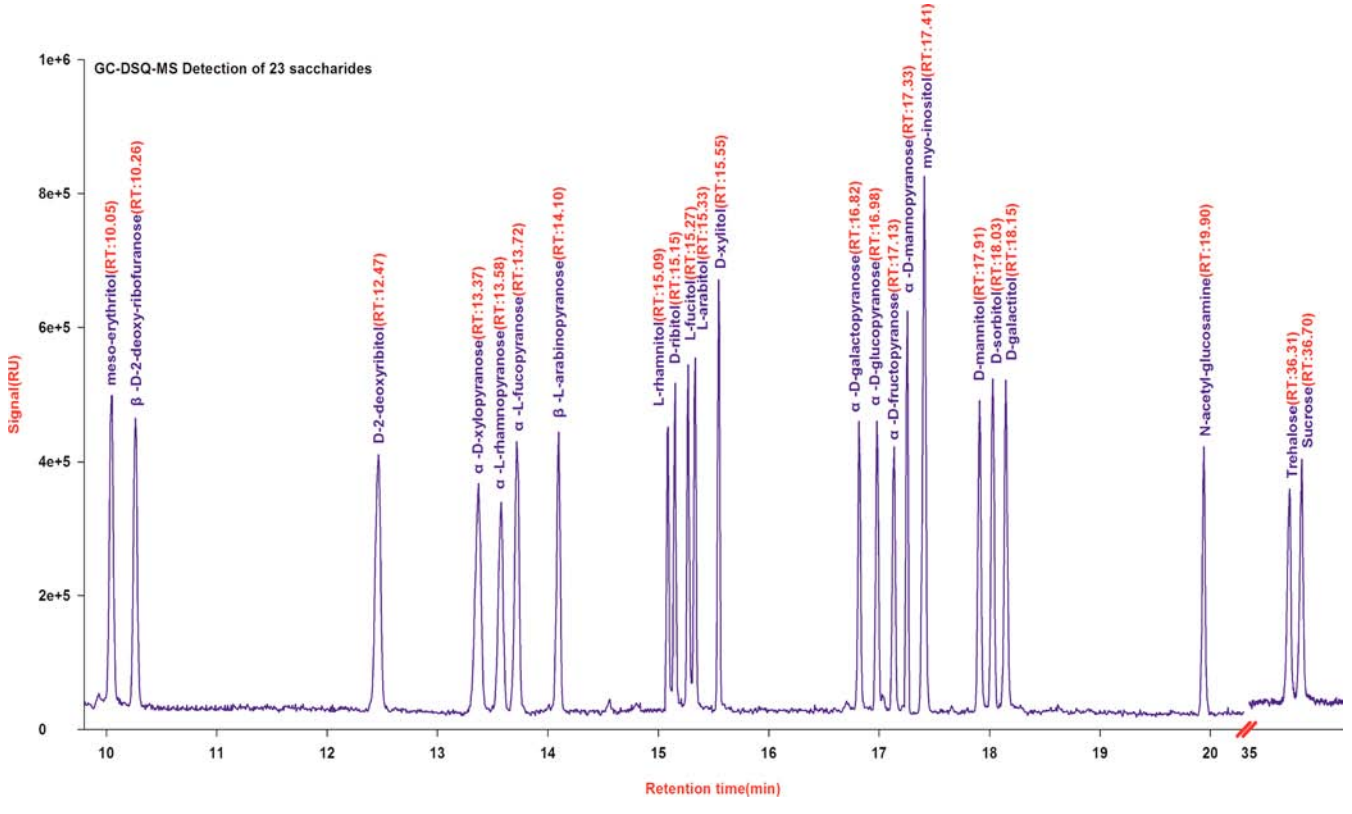

Figure 2. Chromatogram of the total ion current of the acetylated monosaccharide and disaccharide standards.

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will yield two peaks by oximation, and different aldoses and ketoses can yield the same alditol upon reduction, leading to the inability to detect some important monosaccharides.

In fact, the mutarotation of monosaccharides is directly related to sugar structures, temperature, and chemical properties of solvent.[13,14] In sugar gas chromatographic analyses, high temperature always leads to occurrence of mutarotation.[13,14] For example, derivatization techniques such as reduction and oximation often require a high temperature (80−90 °C) to ensure a complete reaction.[12] In addition, solvent properties significantly affect the sugar structure. For example, α-D-glucopyranose undergoes spontaneous transformations upon dissolution in water at 31 °C. On the basis of the ^{13}C NMR spectroscopy results, the compositions of the transformed sugar include α-pyranose (38%), β-pyranose (62%), β-furanose (0.26%), and a chain structure (0.01%). Anomeric ring changes generally occur in prototropic solvents (which have acid and base functions in the same molecule or in a mixed solvent); however, these changes occur less often in acidic or basic solvent systems and are completely absent in inert solvents.[13,14] Thus, the configuration of α-pyranose can be retained if the crystal is dissolved in the inert solvent Me_2SO.

The research inspired us to develop an optimal derivatization approach for saccharide analysis, in which the Me_2SO/1-MeIm system was used for solubilizing carbohydrates in acetic anhydride esterification reactions but also inhibiting the well-known oxidation that occurs.[17,18] The new method avoids many of the former problems of reduction and oximation procedures as 23 kinds of saccharides yielded unique retention times and chromatographic peaks (Figure 2). These acetylated derivatives included 8 kinds of aldose, 1 kind of ketose, and 11 kinds of alditol, which indicates aldose, ketose, and alditol can be analyzed by GC-MS.

Moreover, to date, there are many pretreatments and extraction approaches for saccharide analysis from biological tissues.[1,6,17,19] Some approaches for sugar determination are controversial. For example, oven-drying for biological tissues may lead to carbohydrate degradation.[1,19] Current research about comparisons of these approaches has not been reported. In this study, Me_2SO as the solvent of extraction and acetylation in the newly developed method was compared to the traditional methods, and an orthogonal test was used according to L_9 (3^3) orthogonal design for pretreatments, extraction, and derivatization approaches to find the most efficient and convenient combination for saccharide detection (Table 1).

MATERIALS AND METHODS

Plant Materials. *Populus tomentosa* Carr. and *Ginkgo biloba* L. were grown under ambient conditions on the campus of Beijing Forestry University for approximately 10 years. Young leaves were collected in May 2010 and immediately used for saccharide extraction. Leaves of *Adiantum capillus-veneris* L. were taken from the Beijing Botanical Garden of the Institute of Botany (Chinese Academy of Sciences). *Oryza sativa* L. from a natural population of japonica cultivar was a gift from *Huang Jin Qin*, the Institute of Botany (Chinese Academy of Sciences). *Arabidopsis thaliana* L. was cultured hydroponically in growth chambers as previously described.[8] The rosette leaves were harvested from 4-week-old plants for soluble saccharide extraction. Fresh corn, apple, and cucumber were commercial products from Shengxi market in the Haidian District of Beijing.

Table 1. Orthogonal Layout Design

level	pretreatment (A)	extraction (B)	derivatization (C)
1	freeze-dry	methanol/water, (4:1)	oximation and acetylation
2	oven-dry	Me_2SO	reduction and acetylation
3	fresh sample	methanol/chloroform/water, (12:5:3)	acetylation in Me_2SO/1-MeIm

Experimental Reagents and Solutions. Among the saccharides, about 23 compounds are major soluble saccharides in tissues of a wide range of plant species.[20] Therefore, 23 standards of these saccharides were prepared as comparison controls for the GC-MS analysis: *meso*-erythritol, 2-deoxy-β-D-ribose, α-D-xylopyranose, α-L-rhamnopyranose, α-L-fucopyranose, β-L-arabinopyranose, D-ribitol, D-xylitol, α-D-galactopyranose, α-D-glucopyranose, α-D-fructopyranose, α-D-mannopyranose, *myo*-inositol, D-mannitol, D-sorbitol, N-acetyl-D-glucosamine, trehalose, and sucrose.

Hydroxylamine hydrochloride and 1-methylimidazole were purchased from Sigma-Aldrich China (Shanghai, China). 2-Deoxy-D-ribitol, L-rhamnitol, L-fucitol, L-arabinitol, and D-galactitol were prepared by the reduction of corresponding monosaccharides with $NaBH_4$. Methanol, $NaBH_4$, chloroform, acetic anhydride, and dichloromethane (AR grade) were purchased from Tianjin Chemical Co. (Tianjin, China).

Apparatus. A quadrupole MS system (Trace-GC Ultra) connected to a Trace-DSQ Mass Selective Detector (Thermo Finnigan, San Jose, CA, USA) was used in the analysis of acetyl derivatives on a DB-17 MS fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent USA) and with (EI) ionization of full scan and selected-ion monitoring (selected ion ± 1.0 mass unit). The oven temperatures were programmed as follows: initiation at 100 °C, then gradually ramped to 190 °C (12 °C/min), and held for 6 min; 250 °C (30 °C/min) and held for 6 min; ramped to 280 °C (40 °C/min), and held for 10 min. The temperature at the injector port was 250 °C, and that at the detector port was 260 °C. Helium was used as the carrier gas at a constant flow mode with the rate of 1.0 mL/min. An Xcalibur 2.0 workstation was used for data acquisition and quantitative data processing.

Authentic standards of 23 soluble saccharides were used to determine the retention times of each component by full scan mode. Selective ion monitoring (SIM) mode was used to detect target compounds from plant samples, main fragment ion m/z as follows: 1, *meso*-erythritol (RT = 10.05 min, m/z 145, 115, 103); 2. 2-deoxy-β-D-ribose (RT = 10.26 min, m/z 81, 98, 141); 3, 2-deoxy-D-ribitol (RT = 12.47 min, m/z 159, 103, 117); 4, α-D-xylopyranose (RT = 13.37 min, m/z 128, 170, 157); 5, α-L-rhamnopyranose (RT = 13.58 min, m/z 115, 157, 142); 6, α-L-fucopyranose (RT = 13.72 min, m/z 115, 157, 142); 7, β-L-arabinopyranose (RT = 14.10 min, m/z 128, 170, 115); 8, L-rhamnitol (RT = 15.09 min, m/z 128, 170, 115); 9, D-ribitol (RT = 15.15 min, m/z 115, 145, 103); 10, L-fucitol (RT = 15.27 min, m/z 128, 170, 115); 11, L-arabinitol (RT = 15.33 min, m/z 115, 145, 103); 12, D-xylitol (RT = 15.55 min, m/z 115, 145, 103); 13, α-D-galactopyranose (RT = 16.82 min, m/z 115, 157, 98); 14, α-D-glucopyranose (RT = 16.98 min, m/z 115, 157, 98); 15, α-D-fructopyranose (RT = 17.13 min, m/z 187, 128, 101); 16, α-D-mannopyranose (RT = 17.33 min, m/z 115, 101, 98); 17, *myo*-inositol (RT = 17.41 min, m/z 168, 115, 126); 18, D-mannitol (RT = 17.91 min, m/z 115, 145, 139); 19, D-sorbitol (RT = 18.03 min, m/z 115, 145, 128); 20, D-galactitol (RT 18.15 min, m/z, 115, 187, 127); 21, N-acetyl-D-glucosamine (RT = 19.90 min, m/z 114, 156, 241); 22, trehalose (RT = 36.31 min, m/z 169, 109); and 23, sucrose (RT = 36.70 min, m/z 169, 109, 211).

Analytical Procedure. *Derivatization of Authentic Standard Soluble Saccharides by the New Method.* Two hundred microliters of authentic standard saccharide solution of Me_2SO, mixed with 150 μL of acetic anhydride and 30 μL of 1-methylimidazole, were added and stirred for 10 min in glass tubes. Six hundred microliters of double-distilled H_2O (ddH_2O) was added to the tubes to remove the excess acetic anhydride. Then 200 μL of CH_2Cl_2 was added to extract the acetylated derivatives. The tubes were centrifuged for 1 min to

Table 2. *F* Values of Seven Saccharides in Variance Analysis

	saccharide						
F value[a]	α-D-galactose	α-D-glucose	*myo*-inositol	D-mannitol	D-sorbitol[b]	trehalose	sucrose
A	9612.238[c]	0.111	0.258	1.766	1.709	0.454	1236.976[c]
B	0.312	0.225	1.954	0.876	2.299	0.343	0.017
C	0.030	1981.964[c]	0.788	5081.287[c]	0.638	0.065	0.330

[a]Meaning of A, B, and C can be seen in Table 1. . [b]D-Sorbitol can be detected only by using C_1 (oximation and acetylation) and C_3 (acetylation in Me_2SO/1-MeIm) approaches. The two sets of data are used for variance analysis. [c]The mean difference is significant at the $P < 0.01$ level.

partition the organic phase. Finally, 1 μL of the lower methylene chloride layer was injected for GC-MS analysis. The derivatization conditions have been optimized, and the results are shown in Figures S1 and S2 in the Supporting Information.

Calibration Curve, Lowest Limits of Detection, and Recoveries. The authentic standard of saccharide solution of Me_2SO was diluted to a set of seven levels of standard solution of soluble saccharides: 0.5, 1, 2.5, 6.25, 12.5, 25, and 50 ng/μL. These solutions were derivatized as described above. Three replications of each level were injected into the GC-MS. To determine the lowest limits of detection of soluble saccharides, lower concentrations of acetylated standard solution of soluble saccharides were prepared at 10, 50, 100, 250, and 500 pg/μL.

Comparison of Three Derivatization Approaches by Standard Monosaccharides. α-D-Glucopyranose, α-D-fructopyranose, D-mannitol, and D-sorbitol, representing aldose, ketose, and alditol, respectively, were selected as sugar standards for comparison of three derivatization approaches (oximation and acetylation, reduction and acetylation, and acetylation in Me_2SO). The alditol acetates were prepared as described by Blakeney et al.[7] The aldononitrile acetates were prepared as described by Abebe et al.,[21] and the locked-ring acetyl derivatives were prepared according to the new method as described above under Derivatization of Authentic Standard Soluble Saccharides by the New Method.

Comparison of Different Pretreatment, Extraction, and Derivatization Approaches for Soluble Saccharides from Plant Tissues. In this paper, commonly used methods of pretreatments, extraction, and derivatization for soluble saccharides from plant tissue (fresh leaves of *P. tomentosa* Carr.) were selected, and an orthogonal test was used according to L_9 (3^3) orthogonal table by three factors and three levels of experimental arrangement to find the most efficient and convenient combination for soluble saccharide detection (Table 1).

Application of the New Approach in the Preparation, Extraction, and Derivatization of Soluble Saccharides from Plant Tissues. Fifty milligrams of fresh plant tissues from the leaves of a plant (*A. thaliana* L.), field crop (*Zea mays* L.), vegetable (*Cucumis sativus* L.), and fruit (*M. domestica* Borkh. cv. Red Fuji) was sampled with a 2 mL tube specific for Fastprep (FP120, Thermal, USA). Five hundred microliters of Me_2SO was added to extract the saccharides with Fastprep shaking 10 times (10 s each time) at 4 °C. The homogenate was agitated for 1 h at 4 °C and then centrifuged at 10000 rpm for 5 min. Two hundred microliters of the supernatant was transferred to a 2 mL tube. Then 30 μL of 1-methylimidazole and 150 μL of acetic anhydride were mixed with the supernatant and then stirred for 10 min. Six hundred microliters of ddH_2O was added to the tube to remove the unreacted acetic anhydride, and the acetyl derivatives were extracted with 200 μL of CH_2Cl_2. One microliter of the lower methylene chloride layer of the organic extract of the reaction mixture was injected into the GC-MS for analysis.

Statistical Analysis. The effects of three different pretreatments, three different extractions, and three different derivatization approaches on yields of derived saccharides and their interactions were evaluated by variance analysis. Software SPSS 10.0 was used for data analysis. Differences between parameter means were considered to be significant when the *P* value of the ANOVA *F* test was <0.05. *F* values in Table 2 indicate the ratio of between-group variability to within-group variability in the assay.

RESULTS AND DISCUSSION

Comparison of Three Derivatization Approaches by Standard Monosaccharides. To elucidate the problems of the oximation and acetylation method and the reduction and acetylation method and the advantages of the newly developed method, α-D-glucopyranose, α-D-fructopyranose, D-mannitol, and D-sorbitol that represent aldose, ketose, and alditol, respectively, were selected as standard sugars for comparison of three derivatization approaches (oximation and acetylation, reduction and acetylation, acetylation in Me_2SO/1-MeIm). The results are discussed in sections below (Figure 1).

Problems in Derivatization by Oximation and Acetylation. *Ketose (D-Fructose).* In the experiment, D-fructose could not be quantified by oximation and acetylation, because the two peaks of the oximated, acetylated derivatives of the *syn* and *anti* isomers of D-fructose could not be separated by a DB-17 MS column[15,16] (Figure 1D).

Problems in Derivatization by Reduction and Acetylation. *Aldose (D-Glucose).* As seen in Figure 1B, D-glucose was reduced to D-sorbitol; thereafter, the peak of acetylated D-sorbitol was used to quantify D-glucose (Figure 1H). However, D-sorbitol is a widely distributed alditol in plant samples and is also quantified as D-glucose, which leads to the failure to quantify D-glucose accurately.

Ketose (D-Fructose). D-Fructose could not be determined by reduction and acetylation because there were two possible ways in which OH groups might be attached at C-2 after reduction, leading to two alditols (D-mannitol and D-sorbitol) (Figure 1E,H).

Alditol (D-Mannitol and D-Sorbitol). D-Mannitol and D-sorbitol are widely distributed alditols in biological tissues, and the accurate determination of the alditols is of great importance for the tolerance research of plants.[9] In this experiment, D-glucose was reduced to D-sorbitol and D-fructose was reduced to D-mannitol and D-sorbitol, which influenced the accurate quantification of endogenous D-mannitol and D-sorbitol in plant samples (Figure 1B,E,H).

Problems of Oximation and Acetylation and of Reduction and Acetylation Were Avoided by Acetylation in Me_2SO/1-MeIm System. *Aldose (D-Glucose).* Because Me_2SO is an aprotic solvent, α-D-glucopyranose retains its unique locked-ring structure and maintains the crystal form in Me_2SO. Thus, the polarity of the locked-ring aldose was different from that of ketose and alditol, and each acetyl derivative preserved its unique single chromatographic peak (Figure 1C).

Ketose (D-Fructose). In the experiment here, α-D-fructopyranose was represented as a ketose. Again, use of Me_2SO allowed the maintenance of the locked-ring structure, and the C-2 epimers did not form. As with the aldose, then, α-D-fructopyranose yielded the unique single chromatographic peak

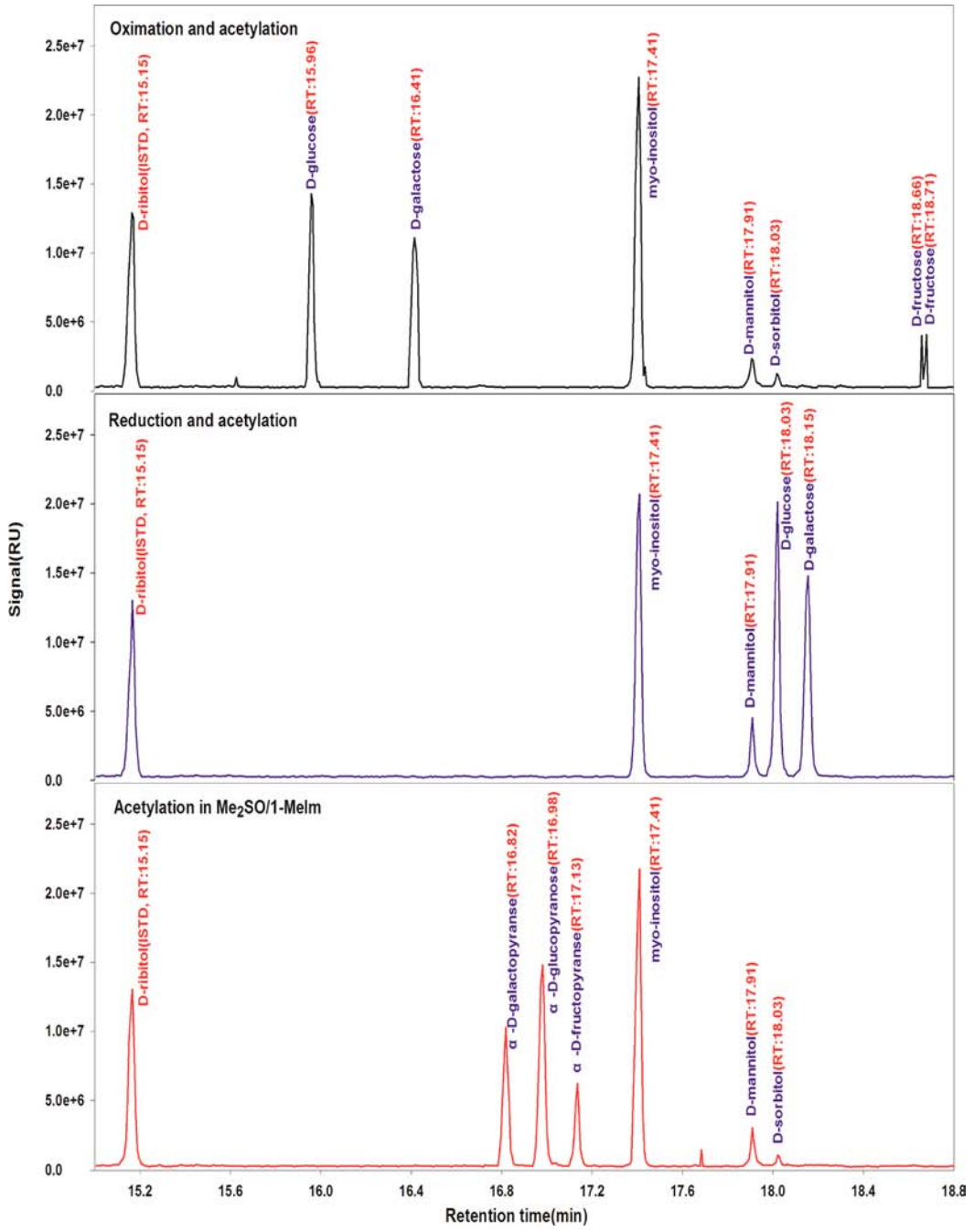

Figure 3. Comparison of three derivatization approaches (oximation and acetylation, reduction and acetylation, acetylation in Me_2SO/1-MeIm) for soluble monosaccharide determination in fresh poplar leaves.

(Figure 1F), suggesting that the new method is the most credible approach for the detection of ketose.

Alditol (D-Mannitol and D-Sorbitol). Because most alditols have a straight-chain structure, the polarity is different from the locked-ring of aldose and ketose. As described above, the new approach maintains the unique, locked-ring structure of aldose and ketose, as well as the straight-chain structure of alditol. The new method, then, allowed for the separation of alditol, aldose, and ketose, leading to easy analysis by GC-MS (Figures 1I and 2).

Comparison of Different Pretreatment, Extraction, and Derivatization Approaches for Soluble Saccharides in Plant Tissues. Leaves of poplar, a modal woody plant, were selected for testing a more accurate, convenient, and environmentally friendly method for sample pretreatment that employed soluble saccharide extraction and their derivatization for saccharide analysis. The results of variance analysis and multiple comparisons of sugars showed that pretreatment A_3 (fresh sample), extraction B_2 (Me_2SO), and derivatization C_3 (acetylation in Me_2SO/1-MeIm) was the best combination for soluble saccharide analysis (Figure 3; Tables 1 and 2; Supporting Information, Table S1).

In comparison of pretreatment of plant tissues, the results showed that high temperature in the A_2 approach significantly affected galactose and sucrose. In contrast, the A_1 and A_3 approaches had no significant effect on all of the saccharides (Table 2; Table S1 in the Supporting Information). When all saccharides are evaluated, A_3 is the best choice among the three

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Table 3. Detection of Standard Soluble Saccharides

no.	name	retention time(min)	resolution	response factor	R^2	recoveries (%)	RSD[a] (%)	theor LOD[b] (mol/L)
1	*meso*-erythritol	10.05	2.21 > 1.5	695.89	0.9941	97.05	1.23	3.1×10^{-8}
2	2-deoxy-*β*-D-ribose	10.26	2.21 > 1.5	256.82	0.9923	95.96	2.67	8.4×10^{-8}
3	2-deoxy-D-ribitol	12.47	46.53 > 1.5	196.11	0.9919	97.17	3.12	1.1×10^{-7}
4	*α*-D-xylose	13.37	15.65 > 1.5	862.90	0.9976	94.24	1.34	2.5×10^{-8}
5	*α*-L-rhamnose	13.58	4.00 > 1.5	237.06	0.9981	91.11	3.45	9.1×10^{-8}
6	*α*-L-fucose	13.72	3.26 > 1.5	2838.50	0.9994	95.49	1.78	7.6×10^{-9}
7	*β*-L-arabinose	14.10	9.44 > 1.5	196.11	0.9996	97.78	0.97	1.1×10^{-7}
8	L-rhamnitol	15.09	11.36 > 1.5	468.97	0.9939	94.11	1.28	4.6×10^{-8}
9	D-ribitol	15.15	2.45 > 1.5	634.49	0.9921	99.04	3.22	3.4×10^{-8}
10	L-fucitol	15.27	4.90 > 1.5	234.48	0.9989	96.83	3.41	9.2×10^{-8}
11	L-arabinitol	15.33	2.67 > 1.5	371.94	0.9902	94.77	2.25	5.8×10^{-8}
12	D-xylitol	15.55	8.35 > 1.5	513.63	0.9955	92.16	1.66	4.2×10^{-8}
13	*α*-D-galactose	16.82	61.95 > 1.5	342.42	0.9949	96.21	2.23	6.3×10^{-8}
14	*α*-D-glucose	16.98	8.00 > 1.5	312.65	0.9991	94.18	3.67	6.9×10^{-8}
15	*α*-D-fructose	17.13	2.00 > 1.5	126.90	0.9945	95.96	2.69	1.7×10^{-7}
16	*α*-D-mannose	17.33	2.10 > 1.5	325.25	0.9934	98.35	3.71	6.6×10^{-8}
17	*myo*-inositol	17.41	10.77 > 1.5	3852.25	0.9965	96.16	4.28	5.6×10^{-9}
18	D-mannitol	17.91	20.41 > 1.5	3922.29	0.9979	96.60	1.43	5.5×10^{-9}
19	D-sorbitol	18.03	4.90 > 1.5	1797.72	0.9947	95.51	2.19	1.2×10^{-8}
20	D-galactitol	18.15	5.71 > 1.5	1135.40	0.9973	96.89	1.45	1.9×10^{-8}
21	N-acetyl-D-glucosamine	19.90	15.909 > 1.5	214.28	0.9995	94.12	1.33	4.1×10^{-8}
22	trehalose	36.31	109.25 > 1.5	12.70	0.9956	98.30	3.48	1.7×10^{-6}
23	sucrose	36.70	3.90 > 1.5	12.70	0.9985	103.25	4.84	1.7×10^{-6}

[a]RSD, relative standard deviation. [b]LOD, limit of detection.

preparation approaches, because the steps of A_1 are more time-consuming compared to those of A_3.

From the analysis of variance (Table 2), we can see that the mean amounts of the three levels of B (extraction method) show no significant difference. However, the extraction liquid of B_1 and B_3 needs to be dried to prevent acetic anhydride from reacting with polar compounds of extraction liquid, a process that can result in the failure of derivatization reaction. Because B_2 uses Me_2SO, an inert solvent, the derivatization reaction is not affected by this process,[15,16] so the best method is B_2 for a plant sample.

In the experiment, six monosaccharides were detected by the C_3 approach, including two aldoses (galactose and glucose), one ketose (fructose), and three alditols (inositol, mannitol, and sorbitol) in fresh leaves of poplar. In contrast, five monosaccharides (except fructose) were determined by C_1, and four monosaccharides (except fructose and sorbitol) were determined by C_2 (Figure 3; Table S1 in the Supporting Information). Therefore, C_3 is the best choice.

Quantitative Analysis and Calibration Curve of Standard Soluble Saccharides. Each of the 23 soluble saccharides had its own standard calibration curve (Table 3). The results showed high linearity in the experimental range of 0.05−50 mg/L. The correlation coefficients for all of the soluble saccharides were 0.9919−0.9996 (n = 3) (Table 3).

Other Advantages of the New Method. *Reduction of the Demand for Temperature and Time for Derivatization of Saccharides.* Conventional derivatization methods such as silylation, oximation, and reduction with subsequent acetylation often need higher temperatures over longer reaction times to ensure completeness.[4] However, the thermal stability of monosaccharides is relatively low. Carbohydrates undergo dehydration to some extent and further degradation such as the well-known caramelization reactions.[3] In the approach presented here, Me_2SO allowed for the maintenance of monosaccharide crystal configurations, and 1-methylimidazole allowed for the acetylation reaction to finish in 10 min at room temperature (Supporting Information, Figure S2). It was a more efficient and faster reaction than the previously reported carbohydrate derivatization for gas chromatography.

High Stability of Derivatization Reagents. In the new approach, unlike silylating agents,[15] 1-methylimidozale and acetic anhydride were stable reagents. Even though some water remained in the reaction solvent, excess acetic anhydride could make the derivatization reaction successful. The stability of sugar derivatives by the new method was determined, and the results indicated that the derivatives were relatively stable (Supporting Information, Table S3).

Environmentally Friendly Derivatization Approach. Unlike oximation or reduction approaches, which use toxic or highly toxic reagents such as pyridine, hydroxylamine hydrochloride, or sodium borohydride, the new method uses less toxic reagents, which is much safer for the operators. In addition, Me_2SO is transparent, colorless, odorless and of low toxicity and is an environmentally friendly, water-soluble compound that can dissolve many compounds. Both the reaction speed and the recovery rate of sugar derivatives are improved in the Me_2SO/1-MeIm system.[22]

Application of the New Method for Detection of Soluble Saccharides in Different Plant Tissues. After the new method had been established using fresh leaves of poplar, the newly developed method was applied in the analysis of soluble saccharides in the leaves of a plant (*A. thaliana* L.), field crop (*Z. mays* L.), vegetable (*C. sativus* L.), and fruit (*M. domestica* Borkh. cv. Red Fuji). Various kinds of soluble monosaccharides of plant tissues, which formerly could not be detected accurately by previous reduction or oximation rections, can be analyzed by this new method. These monosaccharides include *α*-D-fructopyranose, L-rhamnitol, L-arabinitol, D-xylitol, D-mannitol, D-sorbitol, and D-galactitol.

Table 4. Soluble Saccharide Contents in Plant Tissues Analyzed with the New Method

			species, contents (mg/100 g of fresh weight, n = 3)			
no.	name	RT (min)	*Arabidopsis thaliana* L.	*Zea mays* L.	*Malus domestica* Borkh. cv. Red Fuji	*Cucumis sativus* L.
1	*meso*-erythritol	10.05	ND[a]	ND	ND	ND
2	2-deoxy-β-D-ribose	10.26	ND	ND	ND	ND
3	2-deoxy-D-ribitol	12.47	ND	ND	ND	ND
4	α-D-xylose	13.37	0.83 ± 0.03	ND	ND	ND
5	α-L-rhamnose	13.58	0.39 ± 0.09	0.08 ± 0.04	2.02 ± 0.04	0.42 ± 0.05
6	α-L-fucose	13.72	1.01 ± 0.03	ND	0.04 ± 0.02	0.02 ± 0.01
7	β-L-arabinose	14.10	2.00 ± 0.06	0.14 ± 0.02	15.22 ± 0.61	0.70 ± 0.09
8	L-rhamnitol	15.09	6.02 ± 0.20	0.19 ± 0.02	ND	0.09 ± 0.03
9	D-ribitol	15.15	ISTD[b]	ISTD	ISTD	ISTD
10	L-fucitol	15.27	ND	ND	ND	ND
11	L-arabinitol	15.33	19.20 ± 0.62	0.43 ± 0.08	6.03 ± 0.58	1.03 ± 0.06
12	D-xylitol	15.55	0.37 ± 0.04	0.28 ± 0.03	5.99 ± 0.05	0.48 ± 0.03
13	α-D-galactose	16.82	4.01 ± 0.52	0.06 ± 0.04	2.40 ± 0.02	0.46 ± 0.04
14	α-D-glucose	16.98	39.31 ± 1.11	12.25 ± 0.18	561.43 ± 2.23	71.47 ± 0.91
15	α-D-fructose	17.13	8.21 ± 0.21	0.95 ± 0.05	6.35 ± 0.25	0.35 ± 0.05
16	α-D-mannose	17.33	ND	ND	ND	ND
17	*myo*-inositol	17.41	20.04 ± 1.20	0.83 ± 0.22	36.63 ± 0.22	6.23 ± 0.92
18	D-mannitol	17.91	1.27 ± 0.05	0.12 ± 0.04	4.15 ± 0.04	0.12 ± 0.04
19	D-sorbitol	18.03	3.02 ± 0.36	4.79 ± 0.09	121.63 ± 2.04	0.39 ± 0.09
20	D-galactitol	18.15	0.99 ± 0.03	1.21 ± 0.07	1.35 ± 0.25	0.55 ± 0.07
21	N-acetyl-D-glucosamine	19.90	ND	ND	ND	ND
22	trehalose	36.31	ND	ND	ND	ND
23	sucrose	36.70	50.26 ± 1.96	437.6 ± 2.53	12.26 ± 0.05	17.6 ± 1.02

[a]ND, not detected. [b]ISTD, internal standard.

Among the different plant tissues, the differences were mainly reflected in α-D-glucopyranose, α-D-fructopyranose, *myo*-inositol, D-sorbitol, and sucrose (Table 4). Besides fresh plant samples, the method was suitable for almost all kinds of biological samples containing soluble saccharides in many fields, such as food science, chemistry, environmental science, agricultural science, and biology.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

Me_2SO, methyl sulfoxide; 1-MeIm, 1-methylimidazole; GC, gas chromatography; GC-MS, gas chromatography–mass spectrometry; EI, electron impact ionization source; SIM, selective ion monitoring; RT, retention time; LODs, limits of detection; RSDs, relative standard deviations

■ REFERENCES

(1) Sanz, M. L.; Martínez-Castro, I. Recent developments in sample preparation for chromatographic analysis of carbohydrates. *J. Chromatogr., A* **2007**, *1153*, 74–89.

(2) Fuzfai, Z.; Kovacs, E.; Molnar-Perl, I. Simultaneous identification and quantification of the sugar, sugar alcohol, and carboxylic acid contents of sour cherry, apple, and ber fruits, as their trimethylsilyl derivatives, by gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **2004**, *52* (25), 7444–7452.

(3) Rojas-Escudero, E.; Alarcón-Jiménez, A. L.; Elizalde-Galván, P.; Rojo-Callejas, F. Optimization of carbohydrate silylation for gas chromatography. *J. Chromatogr., A* **2004**, *1027* (1–2), 117–120.

(4) Larre-Larrouy, M. C.; Feller, C. Determination of carbohydrates in two ferrallitic soils: analysis by capiliary gas chromatography after derivatization by silylation. *Soil Biol. Biochem.* **1997**, *29*, 1585–1589.

(5) Fox, A. Carbohydrate profiling of bacteria by gas chromatography-mass spectrometry and their trace detection in complex matrices by gas chromatography-tandem mass spectrometry. *J. Chromatogr., A* **1999**, *843* (1–2), 287–300.

(6) Molnar-Perl, I. Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food. *J. Chromatogr., A* **2000**, *891* (1), 1–32.

(7) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113* (2), 291–299.

(8) Persson, S.; Caffall, K. H.; Freshour, G.; Hilley, M. T.; Bauer, S.; Poindexter, P.; Hahn, M. G.; Mohnen, D.; Somerville, C. The *Arabidopsis* irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell.* **2007**, *19* (1), 237–255.

(9) Foster, C. E.; Martin, T.; Pauly, M. Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part II: carbohydrates. *J. Vis. Exp.* **2010**, *37*, 1837.

(10) Nevins, D. J.; English, P. D.; Albersheim, P. The specific nature of plant cell wall polysaccharides. *Plant Physiol.* **1967**, *42* (7), 900–906.

(11) Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340−345.
(12) Knapp, D. R. Part II. Derivatizaion of particular compound types. In *Handbook of Analytical Derivatization Reactions*; Wiley: New York, 1979; pp 338−345.
(13) Pigman, W.; Isbell, H. S. Mutarotation of sugars in solution. I. History, basic kinetics, and composition of sugar solutions. *Adv. Carbohydr. Chem. Biochem.* **1968**, *23*, 11−57.
(14) Isbell, H. S.; Pigman, W. Mutarotation of sugars in solution. II. Catalytic processes, isotope effects, reaction mechanisms, and biochemical aspects. *Adv. Carbohydr. Chem. Biochem.* **1969**, *24*, 13−65.
(15) Andrews, M. A. Capillary gas-chromatographic analysis of monosaccharides: improvements and comparisons using trifluoroacetylation and trimethylsilylation of sugar *O*-benzyl- and *O*-methyloximes. *Carbohydr. Res.* **1989**, *194*, 1−19.
(16) Werner, F.; Clemens, V. S. Syn and anti forms of some monosaccharide *O*-methyl oximes: a ^{13}C-n.m.r. and g.l.c. study. *Carbohydr. Res.* **1979**, *69*, 247−251.
(17) Lu, F.; Ralph, J. Non-degradative dissolution and acetylation of ball-milled plant cell walls: high resolution solution-state NMR. *Plant J.* **2003**, *35*, 535−544.
(18) Albright, J. D.; Goldman, L. Dimethyl sulfoxide-acid anhydride mixture. New reagents for oxidation of alcohols. *J. Am. Chem. Soc.* **1965**, *87* (18), 4214−4216.
(19) Ouajai, S.; Shanks, R. A. Composition, structure and thermal degradation of hemp cellulose after chemical treatments. *Polym. Degrad. Stab.* **2005**, *89* (2), 327−335.
(20) Tan, Y. P.; Li, K.; Hu, L.; Gai, Y.; Jiang, X. N. Fast and simple droplet sampling of sap from plant tissues and capillary microextraction of soluble saccharides for picogram-scale quantitative determiation with GC-MS. *J. Agric. Food Chem.* **2010**, *58* (18), 9931−9935.
(21) Abebe, T.; Guenzi, A. C.; Martin, B.; Cushman, J. C. Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol.* **2003**, *131*, 1748−1755.
(22) Corey, E. J.; Chaykovsky, M. Methylsulfinyl carbanion (CH_3-SO-CH_2^-). Formation and applications to organic synthesis. *J. Am. Chem. Soc.* **1965**, *87* (6), 1345−1353.