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Single-Point Calibration with a Non-linear Detector: Carbohydrate Analysis of Conifer Needles by Hydrophobic Interaction Chromatography–Evaporative Light-Scattering Detection (HIC–ELSD)

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ABSTRACT

An analytical method employing hydrophobic interaction chromatography (HIC) combined with parallel detectors, provided a quantitative method for the determination of soluble carbohydrates in conifer needle tissues. Evaporative light-scattering detection (ELSD), in parallel with atmospheric pressure chemical ionization mass spectrometry (APCI-MS), yielded excellent quantitative and spectral data. Non-linear detector responses of the ELSD were overcome by performing a simple exponential transformation of the detector response data. The transformation

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allowed for single-point calibrations that yielded quantitative results with excellent accuracy and precision. Method recovery and precision were determined from maltose-fortified conifer tissues. Maltose recovery was 101.7% [relative standard deviation (RSD) = 9.74%] for homogenized Douglas-fir (*Pseudotsuga menziesii*) needle tissue fortified at 1.01 mg/g and 107.8% (RSD = 2.47%) for tissue fortified at 10.1 mg/g. Maltose recovery from fortified western redcedar (*Thuja plicata*) tissues (7.60 mg/g) was 93.0% (RSD = 3.0%). The method limit of detection (MLOD) was 0.24 mg/g for maltose in Douglas-fir. The method was employed for the quantitative analysis of soluble carbohydrates in Douglas-fir needles, collected from new and previous-year's growth for 7 weeks after the emergence of new growth (bud break). Needles collected from dormant trees were also analyzed. The concentrations of fructose and glucose were consistently greater in previous-year's growth vs. new and dormant growth.

Key Words: Single-point calibration; Conifer needles; Parallel detector; Sample extraction; Carbohydrates; Evaporative light-scattering detector.

INTRODUCTION

Numerous liquid chromatographic methods have been developed for the analytical separation and detection of carbohydrates in plants and foodstuffs. Chief among these, has been anion-exchange chromatography with pulsed amperometric detection (PAD), as first demonstrated by Rocklin and Pohl.[1] This technique has been successfully applied to the analysis of non-structural carbohydrates in woody plants.[2,3] Refractive index detection (RID) has also been employed in chromatographic analyses of plant extracts, though it is generally accepted that the PAD approach yields improved sensitivity vs. RID and is better suited for gradient elution methods.[4,5]

Chromatographic separation of carbohydrate species has not been limited to the anion-exchange mechanism. Reversed-phase mechanisms and ion-pairing techniques have been employed, as well as hydrophobic interaction chromotography (HIC) separations.[6] We were intrigued by application of HIC for chromatographic analyses of small carbohydrates, because simple mobile phases could be employed that are compatible with both evaporative light-scattering detection (ELSD) and atmospheric pressure chemical ionization mass spectrometric (APCI-MS) detection. Parallel deployment of ELSD and APCI-MS detection was considered a viable approach to yield both reliable quantitative results (ELSD), while also yielding spectral confirmation of analytes during method development (APCI-MS). A similar system has proven useful for high-throughput natural product research.[7]

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Both ELSD and APCI-MS have been employed for detection of carbohydrates in chromatographic systems. APCI-MS detection of carbohydrates in negative ionization mode can be easily realized through a chloride attachment mechanism.[8] Addition of chloroform (0.5–5.0%) to the mobile phase promotes chloride attachment in the ionization source. Methods for the analysis of mono- and oligosaccharides in plant tissues have successfully employed ELSD, with good quantitative results.[9–11] ELSD offers gradient capabilities, good peak shape, and adequate sensitivity.[12] However, one drawback of the ELSD is that detector response is exponential, rather than linear, over concentration ranges of several orders of magnitude.

ELSD detector response is given by the following equation where: A is the detector response (area counts), b is a constant, M is the amount of the analyte (mass or concentration), and x describes the exponential function [Eq. (1)].[13]

$$A = bM^x \tag{1}$$

The exponent (x) is equal to the slope of the line generated by the common treatment of ELSD response data [Eq. (2)].[13] Note, that Eq. (2) fits a linear model. Thus, calibrations can be generated by plotting the log of the detector response vs. the log of the analyte concentration.

$$\log(A) = x\log(M) + \log(b) \tag{2}$$

However, a linear equation may also be produced by performing a $1/x$ exponential transformation of Eq. (1) where: K is a new constant equal to the constant b to the $1/x$ power [Eq. (3)]. Note, that Eq. (3) also fits a linear model but does not contain an intercept. Further, analyte concentration is represented in this equation without an exponent. These characteristics indicate that such a transformation could be used for quantitative analysis vs. single-point calibrations.

$$A^{1/x} = KM \tag{3}$$

In support of a study designed to examine herbivore preferences for woody plants, we developed a chromatographic method for the analyses of non-structural carbohydrates in extracts of conifer needles. Parallel detectors, ELSD and APCI-MS, were employed to yield quantitative and spectral data. A significant aspect of this work was identifying the method of data transformation that could be applied to the ELSD response data to produce a linear function. Furthermore, we sought to demonstrate that this linear function was suitable for quantitative analysis vs. a single-point external standard.

EXPERIMENTAL

Equipment and Supplies

Water, acetonitrile, and chloroform (HPLC Grade) were used to prepare the mobile phase (Fisher Scientific, Fair Lawn, NJ). Chloroform (3%) was added to acetonitrile to produce the organic portion of the mobile phase (Fisher Scientific). Punctilius ethanol was used to prepare standard solutions and the extraction solution (National Distillers and Chemical Corp., NY). Carbohydrate standard solutions were prepared in 1 : 1 ethanol : water. The carbohydrates investigated for this method were fructose, glucose, sucrose, raffinose, inositol, melibiose, maltose, fucose, 2-deoxy galactose, 2-deoxy glucose, and rhamnose (Aldrich Chemical, Milwaukee, WI).

Vacuum-degassed mobile phase was delivered with a binary pump (HP1100, Agilent Technologies, Palo Alto, CA). Injections (5-μL) were made with an autosampler equipped with a 100-μL metering valve (HP1100). Two analytical columns were evaluated for this method. The first was a Hypersil APS-2 100 × 3 mm amino bonded phase column with 5-μm particle size (Thermo Hypersil, Bellefonte, PA). The column ultimately chosen for this method was a 150 × 3 mm BioBasic AX with 5-μm particle size (Thermo Hypersil). The column temperature was maintained at 25°C with a column heater unit (HP1100). Detection was achieved with an ion trap tandem mass spectrometry, equipped with an APCI source (LCQ, Thermo Finnigan Corp., San Jose, CA) and a Sedex 75 ELSD (SEDERE, Alfortville, France). Post-column flow was diverted to the two detectors in a 5 : 1 ratio (ELSD : APCI-MS) with an ASI Series 620 fixed-flow splitter (Analytical Scientific Instruments, El Sobrante, CA). The ELSD output was captured by a HP35900C analog/digital interface (Agilent Technologies).

The mobile phase composition at injection was 10% aqueous, at which time the gradient program was started. At 5 min, the mobile phase was 40% aqueous and was held at that composition for 2 min. During the period of 7–8 min, the mobile phase was returned to the starting composition (10% aqueous). Total runtime was 13 min.

The ELSD temperature was 50°C and the nebulizer pressure (air) was 3.5 bar. The mass spectrometer was operated in the negative ion mode. The vaporizer temperature was 395°C; sheath gas flow 75%; auxiliary gas 0%; corona discharge current 5 μA; and the heated capillary temperature was 215°C. Detection of the carbohydrates was achieved by tandem mass spectrometric isolation of the $[M+^{35}Cl]^-$ parent ion, followed by collision induced fragmentation (30% collision energy; He). The collision products were scanned from 100 to $[M+^{35}Cl]^-$ m/z. Both the full and tandem (ms–ms) mass spectrometric results were used to produce chromatograms.

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A freezer mill (Model 6850, SPEX CertiPrep Inc., Metuchen, NJ) was employed to homogenize the foliage samples, and a vacuum packaging system (Food Saver Professional II, Tilia International, San Francisco, CA) was used to seal samples in disposable bags until analysis. A horizontal mechanical shaker (Eberbach, Ann Arbor, MI) and bench-top centrifuge (Fisher Sci., Pittsburgh, PA) were used in the preparation of sample extracts.

Plant pigments were removed from the extracts with 250 mg graphitized non-porous carbon, 500 mg octadecyl, and 500 mg aminopropyl solid phase extraction (SPE) columns (3-mL reservoir; Supelco, Bellefonte, PA). Plungers from 3-mL disposable syringes were used to force extracts through unconditioned SPE columns (Becton Dickinson and Company, Franklin Lakes, NJ).

Conifer Sample Preparation

Samples were collected from Douglas-fir (*Pseudotsuga menziesii*) seedlings on a weekly schedule, beginning with bud-break (flushing of new growth). At each sampling interval, new growth was removed from three lateral branches from five unique seedlings. Previous-year lateral growth (first internode) was similarly removed from the same five seedlings. A third tissue collection was made from dormant seedlings maintained in a storage cooler at 5°C, using the same sampling protocol. Tissues were collected for 7 weeks. Foliage collections were combined by type (new, previous-year, or dormant) to yield unique composite samples from each week of sampling. Composite samples were retained in vacuum-sealed sample bags and frozen until analysis, at which time needles were removed from lateral stems. The needles were homogenized in liquid nitrogen with an automated freezer mill. Following homogenization, the ground needle material was re-sealed in individual vacuum storage bags, and returned to the freezer until extraction and chromatographic analysis less than 1 week later.

Sample Extraction

Approximately 1 g of homogenized Douglas-fir foliage sample was placed in 50-mL screw-cap culture tubes and the actual masses recorded. A 1 : 1 ethanol : water extraction solution (15 mL) was added to each tube, the tubes were capped, and placed in a horizontal mechanical shaker for 10 min. The tubes were centrifuged and the extracts subjected to SPE clean-up to remove pigments. For clean-up, approximately 2 mL of each extract were loaded onto individual non-porous carbon SPE columns, and the extracts were forced through the SPE columns with 3-mL syringe plungers (as if

the columns were syringe bodies). Extracts were eluted directly into autosampler vials and capped for chromatographic analysis.

This method was also evaluated for the analysis of ground western redcedar (*Thuja plicata*) foliage. The extraction procedures for western redcedar tissues were identical to those described for Douglas-fir tissues, except that the procedure for SPE removal of plant pigments was modified. Because non-porous carbon SPE did not visually remove all pigments, a two-step procedure was employed. First, approximately 2 mL of each redcedar extract were loaded onto individual octadecyl SPE columns and the extracts eluted with a syringe plunger into individual 25-mL glass culture tubes. Each eluate was then loaded onto individual aminopropyl SPE columns and similarly eluted directly into autosampler vials.

ELSD Detector Response Evaluation

Solutions of glucose (115–4440 μg/mL), fructose (45.1–4510 μg/mL), sucrose (44.5–4450 μg/mL), and maltose (50.6–749 μg/mL) were prepared in 1 : 1 ethanol : water. Seven mixed standard solutions of glucose, fructose, and sucrose and six individual standards of maltose were prepared. Each solution was injected in triplicate and the ELSD area response recorded for each analyte. The value of the exponent "x" in Eqs. (1) and (2) was determined by linear regression analysis (proc GLM[14]). As described in Eq. (2), log (analyte concentration) was the independent variable and log (area response) was the dependent variable.

ELSD Single-Point Calibration

Detector response data obtained from the response evaluation solutions were transformed according to Eq. (3), where "x" was the value determined for each analyte during detector response evaluation. Linear regression analyses were performed on the transformed data and slope, y-intercept, coefficient of determination (R^2) was determined for each analyte.[14] Furthermore, the null hypothesis of the y-intercept equal to zero was tested.

Quantitative analyses vs. a single point were conducted with a mixed glucose (703 μg/mL), fructose (714 μg/mL), and sucrose (716 μg/mL) working standard. The working standard solution was injected into the instrument in triplicate and the ELSD detector responses were recorded. Mean area response data was transformed according to Eq. (3), using the appropriate value for "x". Response factors (RF) were calculated by dividing the carbohydrate concentration by the mean transformed area response. Analyte

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responses obtained from chromatographic analysis of the extracts were similarly transformed, and the concentrations determined by multiplying the transformed ELSD response value with the appropriate RF.

Method Evaluation

A maltose fortification solution was prepared in 1 : 1 ethanol : water at a concentration of 50.69 mg/mL. Eight individual Douglas-fir samples were fortified with maltose at each of two fortification levels: 10.1 and 1.01 mg (yielding nominal sample concentrations of 10.1 and 1.01 mg/g). The 16 fortified samples and five controls (no fortification) were extracted and analyzed, according to the procedures described for analysis of Douglas-fir samples. Following the same procedure as described for the analytes, maltose was quantified vs. a 749 μg/mL maltose standard solution. Maltose recovery and precision were determined at each fortification level. Eight redcedar samples were similarly fortified at a nominal maltose concentration of 7.60 mg/g. Recovery and precision were evaluated.

Seven replicates from a single sample of homogenized redcedar tissue were extracted and analyzed for fructose, glucose, and sucrose. Precision data were used to evaluate the homogenization process.

The method limit of detection (MLOD) was determined from Douglas-fir samples fortified with maltose at the low fortification level (1.01 mg/g). The MLOD was defined as the concentration of maltose required to produce a detector response equal to three times the baseline noise (measured peak to peak at the retention time of maltose in unfortified extracts).

Analyses of Douglas-Fir Samples

Samples of new, previous-year, and dormant growth were analyzed according to the procedures described for Douglas-fir tissues. The sampling dates (e.g., 2 May 2003, 9 May 2003, etc.) were converted to a continuous week variable (i.e., 1, 2, 3, etc.), and the quantitative data analyzed as a one-factor analysis of variance with week as a covariate. The two responses investigated were fructose and glucose concentration, with tissue type (new, previous-year, or dormant) as the factor.

Carbohydrates were identified by their retention times vs. external standards and their molecular masses, as determined from mass spectral analyses. The molecular masses of unknown peaks were also recorded. Molecular mass was readily determined from the chromatographic trace of the full ms data and identifying the $[M+Cl]^-$ responses. Masses were confirmed by the $[M-H]^-$

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response produced from the ms–ms event. Individual solutions of insositol, melibiose, raffinose, fucose, 2-deoxy galactose, 2-deoxy glucose, and rhamnose (ca. 750 μg/mL) were prepared and injected into the HPLC for confirmation of unknown carbohydrate identities.

RESULTS AND DISCUSSION

Carbohydrate Chromatography

Separation of carbohydrates can be achieved on silica-based columns with amino bonded phases.[6] Mobile phases typically associated with reversed-phase systems yield separations by an HIC mechanism. This method originally employed an amino column for the analysis of carbohydrates in plant tissues. However, degradation of the stationary phase led to continuously decreasing analyte retention times. Self-hydrolysis of the amino bonded phase is common for separations that employ water in the mobile phase.[15] The BioBasic AX column was chosen for this method because it produced stable retention times.

ELSD Detector Response and Single-Point Calibration

Linear regression of log transformed data was used to determine the exponential value (x) for glucose, fructose, sucrose, and maltose (Table 1). Inspection of the slopes and standard errors suggests that each compound had a unique slope over the ranges of interest. These slope values were used to transform the detector response data according to Eq. (3) (Table 2). The regression data indicated that transformed responses for each compound closely fit the linear model (R^2 were all greater than 0.995). Furthermore, none yielded a significant y-intercept ($p > 0.1$ for each compound). These results indicate that the transformed data properly fit the model given by Eq. (3), and that transformed detector response

Table 1. Linear regression analyses of log transformed analyte concentration and log transformed ELSD response.

Carbohydrate	Range (μg/mL)	R^2	Slope	Standard error
Glucose	115–4440	0.9996	1.60	0.0075
Fructose	45.1–4510	0.9980	1.66	0.0175
Sucrose	44.5–4450	0.9978	1.41	0.048
Maltose	50.6–749	0.9985	1.33	0.013

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Table 2. Linear regression analyses of exponentially transformed data [as given by Eq. (3)].

Carbohydrate	Exponent	Range (μg/mL)	R^2	y-Intercept[a]
Glucose	1.60	115–4440	0.9998	0.114
Fructose	1.66	45.1–4510	0.9967	0.116
Sucrose	1.41	44.5–4450	0.9998	0.363
Maltose	1.33	50.6–749	0.9975	0.176

[a]p-Value for test of y-intercept equals 0.

was proportional to analyte concentration [i.e., the RF were constant and equal to $1/K$ as given in Eq. (3)]. Thus, the use of single-point calibrations was justified for these analytes over the concentration range evaluated.

Method Evaluation

The method provided good recovery and precision of maltose from the plant tissues (Table 3). While the source of positive bias in maltose recovery from Douglas-fir tissues fortified at 10.1 mg/g is not apparent, the negative bias observed in maltose recovery from redcedar samples is likely a product of the two-step clean-up procedure. Inspection of chromatograms produced from the analyses of fortified and control Douglas-fir samples indicate that the matrix probably did not contribute to the maltose response (Fig. 1).

The redcedar sample extracted multiple times for evaluation of the homogenization procedure was found to contain fructose and glucose in similar quantities, but did not contain sucrose. The relative standard deviation (RSD) of the fructose response was 2.8% (mean = 9.76 mg/g), while the glucose RSD was 3.1% (mean = 12.4 mg/g). These results demonstrate that the cryogenic homogenization procedure yielded a very homogeneous sample, and indicate that homogenized tissue samples need not be subjected to replicate analyses.

Table 3. Recovery and precision data for the analyses of maltose in fortified plant tissues.

Sample	Fortification (mg/g)	Recovery (%)	SD	RSD
Douglas-fir	1.01	101.7	9.91	9.74
Douglas-fir	10.1	107.8	2.66	2.47
Redcedar	7.60	93.0	2.8	3.0

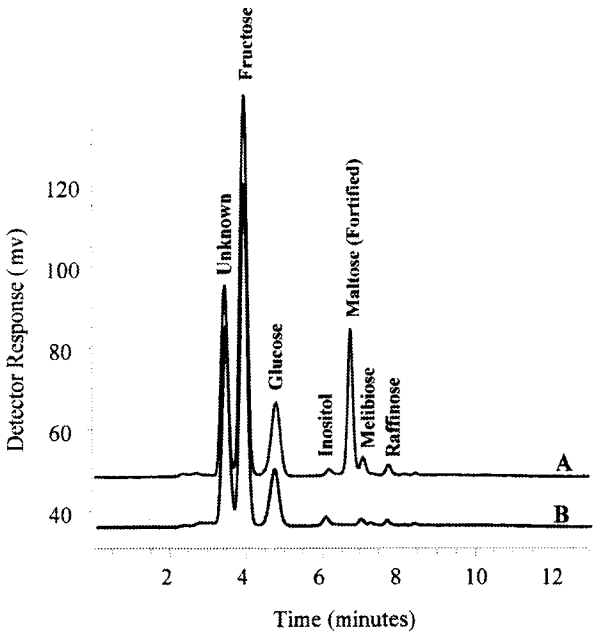

Figure 1. Chromatograms obtained from the chromatographic analysis of Douglas-fir tissue samples. (A) Homogenized needles fortified with maltose at a concentration of 10.1 mg/g. (B) Unfortified needles.

As measured from Douglas-fir samples fortified with maltose, the MLOD was determined to be 0.24 mg/g. A significant decrease in sensitivity (about 15%) results from the instrument configuration that splits a portion of the column effluent to the mass spectrometric detector. Furthermore, early eluting peaks (i.e., fructose and glucose) will have superior sensitivity vs. maltose, owing to improved peak shape. Despite these sources of decreased sensitivity, the calculated MLOD is considerably lower than the values typically observed for glucose and fructose in conifer tissues.

Analyses of Douglas-Fir Samples

Statistical analyses of the fructose and glucose concentration data indicated that the covariate (week) was not significant for either response, while tissue was a significant effect (Table 4). However, the tissue–week interaction

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Table 4. ANOVA table for fructose and glucose concentrations in Douglas-fir tissues collected at seven time intervals beginning with bud-break.

Response	Source	Mean square	*F*-Value	*p*-Value
Fructose	Tissue	121.2	26.3	<0.0001
	Week	12.57	2.73	0.119
	Week–tissue	3.14	0.68	0.5214
	Error	4.61		
Glucose	Tissue	183.4	130.23	<0.0001
	Week	3.31	2.35	0.146
	Week–tissue	5.89	4.18	0.0361
	Error	1.41		

was also significant for the glucose response. These results indicate that fructose concentrations differ among the tissue types, regardless of the week they were sampled (Fig. 2). Mean fructose concentration in previous-year's growth was 21.1 mg/g, while the concentration in dormant tissues was 17.3 mg/g. The lowest fructose concentration was observed in new tissues (12.8 mg/g). Glucose concentrations also differed among tissue types. However, differences were a function of sampling time (Fig. 3). While the glucose concentration was highest in previous-year's growth at each sampling interval

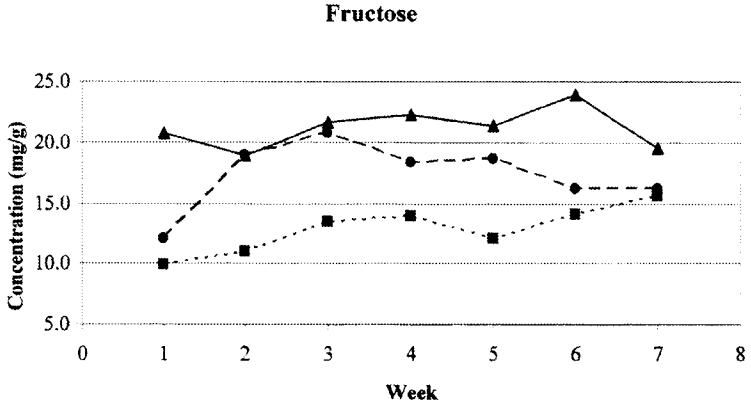

Figure 2. Fructose concentrations in Douglas-fir tissues. Each point represents the analysis of a single composite sample. *Key*: ▲, previous-year's growth; ●, dormant growth; ■, new growth.

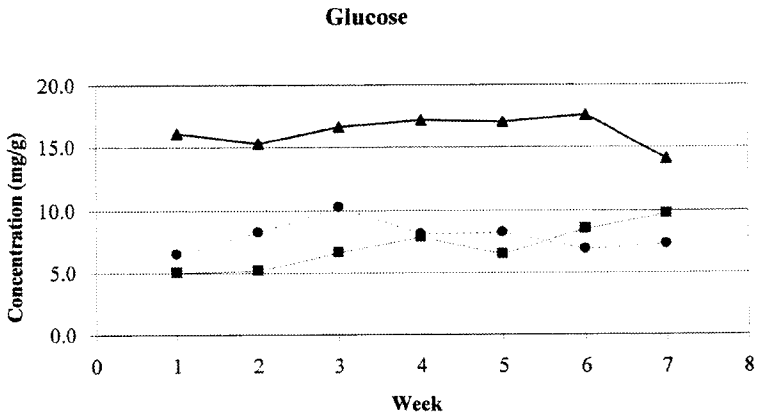

Figure 3. Glucose concentrations in Douglas-fir tissues. Each point represents the analysis of a single composite sample. *Key*: ▲, previous-year's growth; ●, dormant growth; ■, new growth.

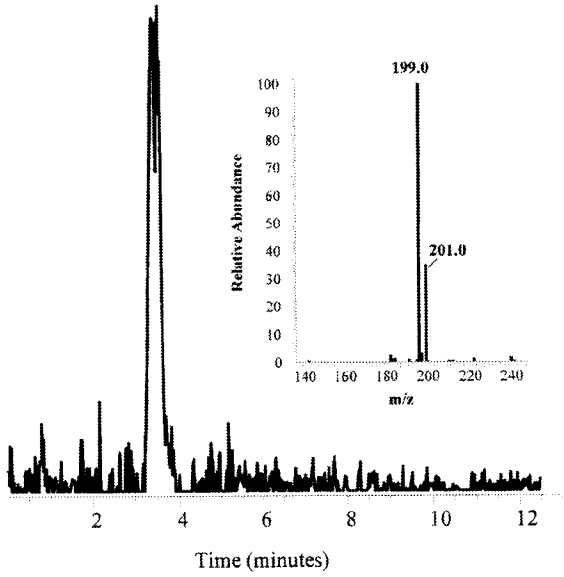

Figure 4. Chromatographic trace of $m/z = 199$ for a representative Douglas-fir extract. The mass spectrum includes the diagnostic $[M+^{35}Cl]^-$ and $[M+^{37}Cl]^-$ responses indicating that the molecular mass of the analyte is 164 (inset).

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(mean = 16.3 mg/g), glucose was higher in dormant tissues versus new growth at bud-break, while higher in new growth versus dominant tissues later in the growing season.

Spectral Confirmation and Identification of Unknowns

In addition to fructose and glucose, inositol, melibiose, and raffinose were identified by their molecular masses in Douglas-fir extracts (Fig. 1). Carbohydrates are easily identified, and masses determined, from the diagnostic $[M+^{35}Cl]^-$ and $[M+^{37}Cl]^-$ mass spectrometric responses (Fig. 4). These identities were subsequently confirmed via comparison to standard solutions. Another unidentified compound with molecular mass of 164 was observed in significant quantities in Douglas-fir extracts (Fig. 4). This mass coincides with a deoxy monosaccharide. However, direct comparisons to 6-deoxy galactose (fucose), 2-deoxy galactose, 2-deoxy glucose, and 6-deoxy mannose (rhamnose) did not yield a confirmatory identification. Rhamnose (retention time ca. 2.9 min) was eliminated from consideration by its lack of a retention time match. However, the other deoxy monosaccharides each matched the retention time of the unknown (ca. 3.4 min). Furthermore, none of these compounds yielded tandem mass spectra that may have enabled differentiation. The precise identity of this compound remains unknown.

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REFERENCES

1. Rocklin, R.D.; Pohl, C.A. Determination of carbohydrates by anion-exchange chromatography with pulsed amperometric detection. J. Liq. Chromatogr. **1983**, *6*, 1577–1590.
2. Wilson, R.; Cataldo, A.; Andersen, C.P. Determination of total nonstructural carbohydrates in tree species by high performance anion-exchange chromatography with pulsed amperometric detection. Can. J. For. Res. **1995**, *25*, 2022–2028.
3. Kimball, B.A.; Nolte, D.L.; Engeman, R.M.; Johnston, J.J.; Stermitz, F.R. Chemically mediated foraging preference of black bears (*Ursus americanus*). J. Mammal. **1998**, *79*, 448–456.

4. Mcbee, G.G.; Maness, N.O. Determination of sucrose, glucose, and fructose in plant-tissue by high performance liquid-chromatography. J. Chromatogr. **1983**, *264*, 474–478.
5. Glyad, V.M. Determination of monosaccharides, disaccharides, and oligosaccharides in the same plant sample by high performance liquid chromatography. Russ. J. Plant Physiol. **2002**, *49*, 277–282.
6. ElRassi, Z. Recent progress in reversed-phase and hydrophobic interaction chromatography of carbohydrate species. J. Chromatogr. A **1996**, *720*, 93–118.
7. Cremin, P.A.; Zeng, L. High-throughput analysis of natural product compound libraries by parallel LC-MS evaporative light scattering detection. Anal. Chem. **2002**, *74*, 5492–5500.
8. Kato, Y.; Numajiri, Y. Chloride attachment negative-ion mass-spectra of sugars by combined liquid-chromatography and atmospheric-pressure chemical ionization mass-spectrometry. J. Chromatogr. B **1991**, *562*, 81–97.
9. Clement, A.; Yong, D.; Brechet, C. Simultaneous identification of sugars by HPLC using evaporative light-scattering detection (ELSD) and refractive-index detection (RI)—application to plant-tissues. J. Liq. Chromatogr. **1992**, *15*, 805–817.
10. Guenu, S.; Seigneuret, J.M.; Dreux, M. Analysis of maltodextrins in plant extracts by LC using evaporative light scattering detection. J. Liq. Chromatogr. **2000**, *23*, 2883–2895.
11. Hernandez, J.L.; Gonzalez-Castro, M.J.; Alba, I.N.; Garcia, C.D. High performance liquid chromatographic determination of mono- and oligosaccharides in vegetables with evaporative light-scattering detection and refractive index detection. J. Chromatogr. Sci. **1998**, *36*, 293–298.
12. Young, C.S. Evaporative light scattering detection methodology for carbohydrate analysis by HPLC. Cer. Foods World **2002**, *47*, 14–16.
13. Young, C.S.; Dolan, J.W. Success with evaporative light-scattering detection. LC GC (North America) **2003**, *21*, 120–128.
14. SAS SAS/STAT version 8. Cary, NC, USA, 1999.
15. Herbreteau, B.; Lafosse, M.; Morinallory, L.; Dreux, M. High performance liquid chromatography of raw sugars and polyols using bonded silica-gels. Chromatographia **1992**, *33*, 325–330.

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