IMPROVED UHPLC-HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR QUANTITATION OF LEVOGLUCOSAN AND ITS ISOMERS

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Biomass burning has a large impact on vegetation on land and is also a significant source of aerosol particles, which can have a large impact on global climate. Levoglucosan (1,6-anhydro-β-D-glucopyranose) is receiving increased attention as a tracer for these biomass burning events. It is formed during the combustive processes of woody material which contains sugars like cellulose[1]. In addition to levoglucosan the structural isomers galactosan and mannosan are formed in varying ratios depending on source material[2]. The most commonly used technique to quantitatively analyze levoglucosan, particularly in geological materials, is gas chromatography/mass spectrometry (GC/MS) after derivatization[3] and this approach has been successfully applied to aerosols and lacustrine sediments. More recently, high performance liquid chromatography (HPLC)/MS methods using electrospray ionization (ESI) were developed. In particular, Gambaro et al. (2008) developed an HPLC-negative ion ESI-MS^2 method using selected reaction monitoring (SRM) with high sensitivity (detection limit 0.3 pg on column (oc))[4]. However, this method was only applied to melted water from ice cores, which contain relatively little matrix compared to typical geochemical samples, while the applied HPLC separation technique yielded virtually no retention for levoglucosan. Hopmans et al. [5] developed a new separation method, based on hydrophilic interaction chromatography (HILIC), using an NH_2 column and a water/acetonitrile/triethylamine mobile phase enabling the detection of levoglucosan in geological samples with complex matrices. In addition, levoglucosan was separated from its structural isomers mannosan and galactosan, although galactosan and mannosan co-eluted. This method was successfully applied to the analysis of levoglucosan in marine sediments up to 130 ka[6]. Here we report the further development of the separation in order to allow the individual quantitation of mannosan and galactosan. In addition we have incorporated deuterated levoglucosan as an internal standard to improve the quantitation and further reduce matrix effects. Finally, we have applied high resolution mass spectrometry to shed light on the identity of the frequently observed unknowns in soils and immature lake sediments.

Analysis were performed using an Ultimate 3000 RS U(ltra)HPLC equipped with autosampler coupled to a Q Exactive Orbitrap MS (Quadrupole-Orbitrap; Thermo Fisher Scientific, US) with heated ESI (HESI) probe. Separation was achieved on 2 Acquity BEH amide columns (Waters Co., USA) in series (2.1 x 150 mm, 1.7 µm particle size) fitted with a 50 mm pre-column of the same material. The compounds were eluted (flow rate 0.2 mL min^{-1}) isocratically with a water/acetonitrile/trimethylamine mobile phase. Detection was achieved in negative ESI mode monitoring *m/z* 150-350 with a resolution of 140,000 ppm. Targeted data dependent MS^2 was performed on any signal within 10 ppm of *m/z* 161.0445 (calculated exact mass of deprotonated levoglucosan). Deuterated (D7) levoglucosan was added to all samples as internal standard. Integrations were performed on mass chromatograms with a 3 ppm mass accuracy and concentrations were corrected for relative response factors of 0.94, 0.60 and 0.96 for

levoglucosan, galactosan and mannosan, respectively, to deuterated levoglucosan as determined with authentic standards.

Levoglucosan and its isomers were detected as their deprotonated molecules $[M-H]^-$ with a detection limit of 5 pg oc (compared to 50 pg oc for Hopmans et al[5]) and a linear range of 4 orders of magnitude. Galactosan and mannosan were now sufficiently separated (resolution of 1.1) to be individually quantitated. Figure 1 shows the mass traces of a methanol extract of soil from a recently burned dune area in Schoorl, The Netherlands. This soil was found to contain 7.2 ug/g levoglucosan, 4.8 ug/g galactosan and 2.6 ug/g mannosan, as well as several additional signals within the 3 ppm mass accuracy range. Interestingly, all of the peaks eluting between 6 and 12 minutes were shown to have nearly identical mass spectra compared to levoglucosan. Although the exact identity of these unknowns has not been determined this finding suggests that several additions structural isomers of levoglucosan may exist, which may have additional biomarker potential. Using this method we analyzed sinking particulate matter and surface sediments in the tropical North Atlantic Ocean to assess the fate of levoglucosan in marine sediments, after which we successfully applied the method to a marine sediment core[7].

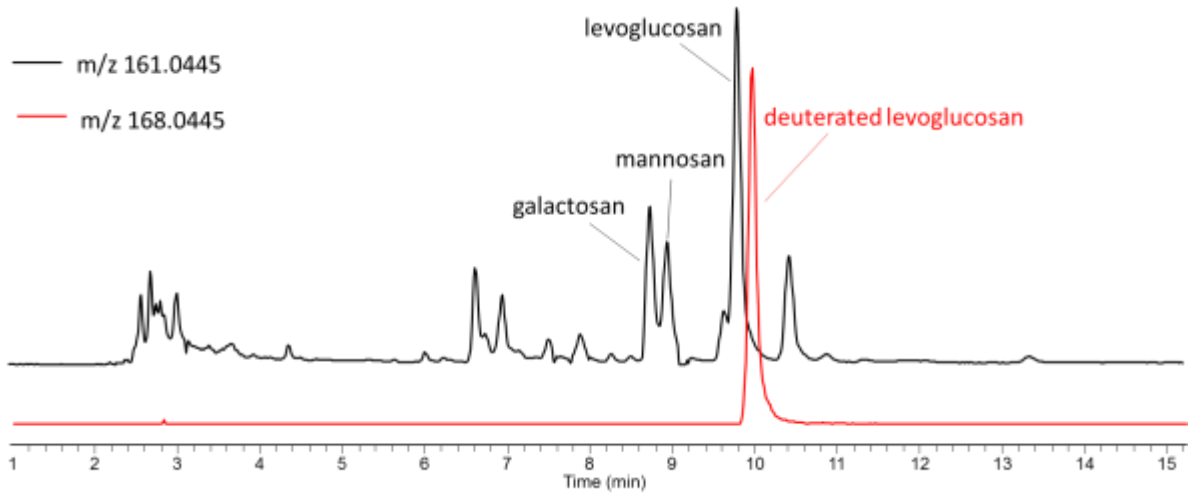

Figure 1 *UHPLC-ESI/MS mass chromatograms (3 ppm mass accuracy) showing levoglucosan, galactosan and mannosan (black line) and the internal standard D7-levoglucosan (red line) in a methanol extract of a soil from a burned dune area (Schoorl, The Netherlands).*

References

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