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# Method for determination of levoglucosan in snow and ice at trace concentration levels using ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry

Chao You [a,b,*], Lili Song [c], Baiqing Xu [a], Shaopeng Gao [a]

[a] Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China
[b] University of Chinese Academy of Science, Beijing 100049, China
[c] Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China

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ABSTRACT

A method is developed for determination of levoglucosan at trace concentration levels in complex matrices of snow and ice samples. This method uses an injection mixture comprising acetonitrile and melt sample at a ratio of 50/50 (v/v). Samples are analyzed using ultra-performance liquid chromatography system combined with triple tandem quadrupole mass spectrometry (UPLC-MS/MS). Levoglucosan is analyzed on BEH Amide column (2.1 mm × 100 mm, 1.7 um), and a Z-spray electrospray ionization source is used for levoglucosan ionization. The polyether sulfone filter is selected for filtrating insoluble particles due to less impact on levoglucosan. The matrix effect is evaluated by using a standard addition method. During the method validation, limit of detection (LOD), linearity, recovery, repeatability and reproducibility were evaluated using standard addition method. The LOD of this method is 0.11 ng $mL^{-1}$. Recoveries vary from 91.2% at 0.82 ng $mL^{-1}$ to 99.3% at 4.14 ng $mL^{-1}$. Repeatability ranges from 17.9% at a concentration of 0.82 ng $mL^{-1}$ to 2.8% at 4.14 ng $mL^{-1}$. Reproducibility ranges from 15.1% at a concentration of 0.82 ng $mL^{-1}$ to 1.9% at 4.14 ng $mL^{-1}$. This method can be implemented using less than 0.50 mL sample volume in low and middle latitude regions like the Tibetan Plateau.



## 1. Introduction

Biomass burning emissions contribute to about half of the carbonaceous aerosols all over the world [1]. The chemical composition inventory can provide detail source identification of the carbonaceous aerosols. Monosaccharide anhydrides (MAs) such as levoglucosan can be adopted as specific molecular tracers for biomass burning aerosols, because they can only be generated by the degradation of cellulose and hemicellulose when the burning temperature is higher than 300 °C [2]. MAs can remain stable for long periods of time, with only negligible degradation in sediment conditions [3,4], which further extends applicability of MAs in historical biomass burning studies.

Snow and ice samples from high latitude/altitude glaciers can provide important information about past fire regimes [3,5,6]. Levoglucosan and its isomers mannosan and galactosan can be used as ideal markers in biomass burning studies of snow and ice [3,5–7]. Several methods have been developed for determination of MAs in snow and ice samples [3,5,7,8]. The gas chromatography based methods need extreme dehydration conditions, which requires a long time for sample preparation [9]. The extreme dehydration conditions reduced the recovery and precision for handling trace concentration of levoglucosan in aqueous samples [5,7]. The high-performance liquid chromatography (HPLC) based method was used as an alternative to overcome these issues. The HPLC with triple quadrupole tandem mass spectrometry (HPLC-ESI-MS/MS) method with low LOD and high recovery ensured accurate results for detecting levoglucosan in Arctic and Antarctic ice [3,4,6]. However, samples from middle and low latitude regions (e.g. the Tibetan Plateau (TP)) usually contain much more complex organic components like the glycose and sugar alcohols besides MAs [5,7]. The method for polar ice showed very poor performance for levoglucosan due to matrix interferences when applied to samples from Tibetan glaciers [8]. A HPLC method with an estimated LOD of 10 ng $mL^{-1}$ was reported for the Tibetan ice cores [8]. However, evidence showed that the levoglucosan concentration was at about 1 ng $mL^{-1}$ level even in regions strongly affected by biomass burning emissions [5,7]. Due to the large quantity of insoluble particles in samples from low and middle latitude

* Corresponding author at: Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China.
*E-mail address:* youchao@itpcas.ac.cn (C. You).

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glaciers [10], direct injection without any pretreatment in previous methods [3,8] is harmful to the HPLC system and chromatographic columns. Accurate quantification of levoglucosan in middle and low latitude glacier snow and ice samples can provide important information for understanding regional biomass burning regimes, which is critical for understanding the relationship of biomass burning, climate change and human activities. Therefore, it is necessary to develop a rapid and effective method for determination of levoglucosan at trace concentration levels in complex matrix snow and ice for regions like the TP.

In this study, a method based on UPLC-MS/MS for determination of levoglucosan in snow and ice is reported. The preparation process and instrument conditions are specific for complex matrix samples from low and middle latitude glaciers.

## 2. Material and methods

### 2.1. Chemicals

HPLC gradient grade acetonitrile (ACN) was obtained from Fisher Scientific (U.S.A.). HPLC gradient grade methanol for standard stock solutions was obtained from J.T.Baker (U.S.A.). HPLC grade ammonium hydroxide (10%) was obtained from Mreda (U.S.A.). Ultrapure water was obtained from a Milli-Q ultrapure water system (U.S.A.). Standard levoglucosan (1,6-anhydro-β-D-glucopyranose, 99%) was obtained from Sigma-Aldrich (St. Louis, U.S.A.), mannosan (1,6-anhydro-β-D-mannopyranose, 98%) was obtained from TRC (Toronto, Canada), and glactosan (1,6-anhydro-β-D-galactopyranose 97%) was obtained from J&K (Pforzheim, Germany). Individual standard stock solutions were prepared using methanol at a concentration of 1000 μg $mL^{-1}$, and progressively diluted by ultrapure water for use. Standard stock solutions were stored in dark conditions at a temperature of 4 °C.

### 2.2. Sample preparation

A total of 61 snow samples were collected from different Tibetan glaciers. Fresh snow samples were compacted in pre-cleaned PET bottles and were kept frozen at a temperature of −20 °C before analysis [7]. Ice samples were obtained from the Zangsegangri ice core. Ice core sections were stored in a freeze storeroom at a temperature of −20 °C. Previous studies indicated that samples stored under these conditions displayed no apparent degradation of levoglucosan for at least several years [3,6]. Sample preparation was carried out in a cold ultraclean room at −18 °C. The outer part of each sample was scraped using a pre-cleaned scalpel, and one portion of the inner part was used for levoglucosan analysis. Fifty ice samples were selected at different depths for levoglucosan analysis in this study. Samples were melted at a room temperature (about 15 °C) in a fume cupboard before analysis, and shaken for 5 min. A sample volume of 0.50 mL of each sample was extracted and filtered by polyether sulfone (PES) filter. The filtrate was collected in a 2.00 mL sample vial, and 0.50 mL ACN was added to each sample before analysis.

### 2.3. Instrumentation

Sample analysis was performed by a Waters Acquity UPLC system (USA) in reversed phase mode. The autosampler was thermostatically controlled at a temperature of 15 °C. A BEH VanGuard Pre-column (2.1 mm × 5 mm, 1.7 um, Waters, USA) was used to protect the chromatography column. For the chromatographic analysis, 5.00 uL of each sample was injected onto a BEH Amide column (2.1 × 100 mm, 1.7 um, Waters, USA). The column temperature was set to 40 °C. The mobile phase comprised ultrapure water with 0.1% $NH_3H_2O$ (mobile A) and ACN (mobile B). Gradient elution was employed at a flow rate of 0.20 mL $min^{-1}$, and the gradient program was shown in Table 1. The retention time of levoglucosan was 1.41 min, and the analytical process lasted 7.00 min in total (Fig. 1).

**Table 1**
Detailed gradient flow program for the UPLC system.

| Time (min) | Mobile A/mobile B | Flow rate (mL $min^{-1}$) | Curve |
|---|---|---|---|
| initial | 65/35 | 0.20 | 6 |
| 0.25 | 65/35 | 0.20 | 6 |
| 0.50 | 50/50 | 0.20 | 9 |
| 7.00 | 50/50 | 0.20 | 6 |

The Acquity triple quadrupole mass spectrometer (TQD) equipped with a Z-spray electrospray ionization (ESI) source was used for determination of levoglucosan in this study. Data were collected in negative ion mode by multiple reactions monitoring (MRM), and the ESI source block temperature was 150 °C. The optimal MS conditions of the mass spectrometers were as follows: source voltage 3.00 kV; source desolvation temperature 500 °C; source gas flow desolvation 800 L $h^{-1}$; cone gas 50 L $h^{-1}$. The ion transition *m/z* 161/101 was used for quantification of levoglucosan in samples. The data were collected and analyzed by Masslynx 4.1 software developed by Waters company.

### 2.4. Method validation

The method was validated following recommendations of ICH and some recent scientific publications relating to the analytical process [3,9,11–14]. During the method validation, LOD, linearity, recovery, repeatability and reproducibility were evaluated using standard additions method. Special attention was paid to evaluating the “matrix effect”, because different Tibetan glaciers might have inconsistent matrix conditions and we did not get a commercial applicable isotope labeled levoglucosan for internal calibration. A snow sample (named KKSL-G) reported with no levoglucosan in our previous study [7] was used as real matrix procedural blanks. The standard addition method was used for evaluating the matrix effect in this study [15], and levoglucosan standard solutions at known concentrations were added into samples after filtering.

## 3. Results and discussion

### 3.1. Optimization of the instrumental performance

The ESI-MS conditions were optimized by infusing single standard of three isomers in both positive and negative ionization mode. No signal was recorded under different ion source parameters in positive mode. Highly abundant analytes signals were detected in negative mode, and ion transitions of the three isomers were selected using single standard solutions by direct infusion at a concentration of 100 ng $mL^{-1}$ into the ion source of the mass spectrometer (161/71 and 161/101 for levoglucosan, 161/59 and 161/101 for galactosan, 161/85 and 161/101 for mannosan). The effect of various ESI-MS parameters such as ESI source temperature (100–200 °C), source voltage (2.5–4.5 kV), source desolvation temperature (300–650 °C) and desolvation gas flow rate (600–1000 L $h^{-1}$) was studied.

C18 columns have usually been used for HPLC determination of levoglucosan in snow/ice [3,8]; however, very poor chromatographic response intensity was observed when the Acquity BEH C18 column (Waters, 2.1 × 100 mm, 2.1 × 75 mm and 2.1 × 50 mm,