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Gas chromatography–mass spectrometry-based trimethylsilyl-alditol derivatives for quantitation and fingerprint analysis of *Anemarrhena asphodeloides* Bunge polysaccharides

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ABSTRACT

Here we report a novel approach using gas chromatography mass spectrometry (GC−MS)-based trimethylsilyl-alditol (TMSA) derivatives for simultaneous baseline separation and detection of 8 neutral saccharides and 2 uronic acids within 25 min. Using mild alkaline conditions to dissolve the sample in advance significantly increased both the detection sensitivity and sample stability of uronic acids because of occurrence of de-lactonization, whereas no obvious effects were observed for neutral saccharides. Sodium borohydride reduction of the carbonyl group of aldoses and the subsequent formation of TMSA derivatives simplifies GC−MS chromatograms by producing a single peak for each derivatized sugar. The effects of both reaction temperatures and solvent ratios between HMDS and TMCS on formations of TMSA derivatives were also investigated. The established GC−MS method was successfully applied for quantitation and fingerprint analysis of polysaccharides from the plant *Anemarrhena asphodeloides* Bunge. A comparative analysis of *A. asphodeloides* polysaccharides was further performed between TMSA and other four types of derivatizations. The results showed that GC−MS analysis based on precolumn TMSA derivatization coupled with fingerprint analysis is a comprehensive and effective technique for qualitative and quantitative analysis of plant polysaccharides from traditional Chinese medicines.

1. Introduction

A variety of chromatographic techniques such as capillary electrophoresis (CE) (Monnig, Kennedy, & Chem, 1994; Sutton, Sutton, & Stalcup, 1997), high-performance liquid chromatography (HPLC) (Kuang et al., 2011, You et al., 2009), and high performance anion exchange chromatography (HPAEC) (De Ruiter, Schols, Voragen, & Rombouts, 1992; Morales, Corzo, & Sanz, 2008) were previously considered as useful tools for the determination of carbohydrates. Although existing techniques are increasingly mature and gradually improving, they still face many problems and challenges. The HPLC-UV and CE-UV methods are too restrictive and cannot be used for the trace quantification of carbohydrates (Emerick, Oliveira, Belaz, & Deoliveira, 2010; Miah, Iqbal, & Lai, 2012). HPAEC can achieve more satisfactory separation than HPLC techniques, but epimerization and degradation can be issues at high pH (Gangola, Jaiswal, Khedikar, & Chibbar, 2014). Because carbohydrates encompass a number of homologues with very similar structures (Suzuki, Kelly, Locke, Thibault, & Honda, 2003), carbohydrate analysis inevitably requires high-resolution separation and determination techniques.

With the progress of chromatographic techniques, gas chromatography (GC) was developed for carbohydrate analysis, which shortened the analysis time and provided high resolution and sensitivity separation (Rodríguez-Sánchez, Soria, Ruiz-Matute, & Sanz, 2013). Although GC and GC–MS are excellent techniques for the analysis of carbohydrates, the preparation of an adequate derivative is necessary. Methyl ethers, acetates, trifluoroacetates, trimethylsilyl ethers (TMS), TMS oximes and diethyl dithioacetal have been used for carbohydrate determinations (Cremese et al., 1998; Horváth & Molnár-Perl, 1997; Ruiz-Matute, Hernández-Hernández, Rodríguez-Sánchez, Sanz, & Martínez-Castro, 2011; Sweeley, Bentley, Makita, & Wells, 1963; Wunschel et al., 2011). However, some methods were not optimal and resulted in low derivatization yields, complicated chromatograms, and time consuming and inseparable chromatographic peaks (Akiyama, Yamazaki, & Tanamoto, 2011; Bárez, Garcia-Villanova, Garcia, & Paramás, 1999; Pitthard & Finch, 2001). In contrast, trimethylsilyl-alditol (TMSA) derivatives have been used for direct analysis of natural sugar alcohols in plant matrixes (Medeiros & Simoneit, 2007). To the best of our knowledge, however, an analytical GC–MS method based on TMSAs has not yet been reported for the determination of monosaccharides.

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Rhizoma Anemarrhenae (zhi mu in Chinese), the dried rhizome of *Anemarrhena asphodeloides*, is a well-known traditional Chinese medicinal herb according to the 2015 edition of the Chinese pharmacopoeia. It has been widely used in China to remove heat, quench fire, promote the production of body fluid and relieve dryness syndrome (Committee, 2015). Recently, some reports have demonstrated that the *A. asphodeloides* polysaccharide decreased the blood glucose in rats with alloxan-induced hyperglycosemia and increased the content in rats with alloxan-induced hypoglycemic (Liu et al., 2012; Zhang et al., 1999). Kiyohara et al. reported that an immunomodulating glucomannan against immunocompetent cells of intestinal Peyer's patches was isolated from the rhizomes of *A. asphodeloides* (Kiyohara, Matsuzaki, & Yamada, 2013). In our previous studies, *A. asphodeloides* polysaccharides were confirmed to possess obvious anti-inflammatory, immunomodulatory, and laxative effects (Lei, Dong et al., 2015; Lei, Zhang et al., 2015).

As a part of a continuing research program, this study reports a novel approach using GC−MS in terms of precolumn TMSA derivatization for simultaneous baseline separation and detection of four groups of positional isomers within 25 min. The established GC−MS method was then successfully applied for quantitation and fingerprint analysis of *A. asphodeloides* polysaccharides.

2. Experimental

2.1. Materials and reagents

Ten batches of *A. asphodeloides* roots as the authentic source (Anhui, China) were provided by First Affiliated Hospital of Heilongjiang University of Chinese Medicine and named as S1-S10. Six batches of *A. asphodeloides* roots as the foreign source from Hebei, named as S'1-S'6, were purchased at the Harbin medical market. D-galacturonic acid (GalUA), D-glucuronic acid (GlcUA), D-fucose (Fuc), L-rhamnose (Rha), D-glucose (Glc), D-galactose (Gal), D-mannose (Man), L-arabinose (Ara), D-ribose (Rib), and D-xylose (Xyl), hexamethyl disilazane (HMDS) and trimethylchlorsilane (TMCS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA) and anhydrous pyridine were purchased from Merck (Zdarmstadt, Germany). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). All other chemicals were of highest analytical grade.

2.2. Preparation of polysaccharides from A. asphodeloides

The 10 batches of dried sample materials (each, 3 g) were immersed in 30 mL distilled water and then ultrasonicated for 45 min at 60 °C and 70 kHz. The filtrate of the obtained extract was condensed under vacuum to syrup and precipitated with 75% ethanol. After standing for 24 h at 4 °C, the precipitate was collected and washed with anhydrous ethanol, and then dried. The residue was re-dissolved in water, and then deproteinated 3 times using 5:1 chloroform-*n*-butanol. The resulting aqueous fraction was extensively dialyzed (cut off M_w, 3500 Da) against tap water for 24 h and distilled water for 24 h. The macromolecular remains after dialysis was lyophilized to yield *A. asphodeloides* polysaccharide extracts (each, for about 270 mg).

2.3. Complete acid hydrolysis of A. asphodeloides polysaccharides

The crude polysaccharides (6 mg) were taken up in 2 M TFA and hydrolysed in capped glass vials for 2 h at 110 °C. After hydrolysis, the hydrolysates were washed with methanol and evaporated to dryness for several times to remove the residue of TFA. Then, 1.0 mL distilled water was added to the dried samples, ultrasonicated for 1 min, the solution centrifuged for 5 min at 12,000 rpm in a tabletop centrifuge, and the supernatant was transferred to 2 mL of centrifuge tube, diluted with deionized water and stored in 4 °C.

2.4. Derivatization of monosaccharides

Preparations of TMSA derivatives included several steps as followed. The mixed monosaccharide standards were firstly treated in the same manner as those of the polysaccharides. The resulting sample was mixed with 1 mL of 0.5 mol/L NH_4OH and maintained at room temperature for 10 min. Then, 10 mg $NaBH_4$ was added, the reaction vessel was tightly closed with a screw cap, and the residue in the vial underwent a standing reaction for 1.5 h at 25 °C. Then, the residue was neutralized with acetic acid to remove the residual $NaBH_4$ and was then evaporated to dryness. The dried product was added to 100 μL of pyridine, followed by HMDS and TMCS (3:1) heated for 30 min in a 70 °C water bath, and then was extracted using a volume of water. The solution was directly filtered through a 0.22-μm membrane before GC–MS analysis.

Methods for preparations of TMS derivatives, alditol acetates and dithioacetal TMS were as described in literatures (Jaddou & Alhakim, 1980; Pitthard & Finch, 2001; Rumpel & Dignac, 2006). The method for preparations of improved TMS- oximes (TMSO) was as described in literatures (Li & Chang, 1988; Xia et al., 2017). Briefly, the carbohydrates were dissolved in 100 μL of pyridine, and then 1.6 mL of derivatization reagent (hydroxylamine hydrochloride: pyridine: HMDS: TFA = 5:0.5:1:0.1) was added into the reaction vial. The resulting solution was gentle swirling and kept for 30 min at 70 °C water bath. The solution was directly filtered through a 0.22 μm membrane before GC–MS analysis.

2.5. GC–MS apparatus and conditions

GC–MS analyses were performed on an Agilent 7890 A GC system equipped with a 5975C EI-MS system (Agilent Technologies, CA, USA). The chromatographic separation was conducted on a DB-5 silica capillary column (60 m × 0.25 mm × 0.25 μm) using the following different temperature profiles. The temperature condition on TMSA derivatization was used as follows: 180 °C for 0 min, 180–190 °C at 4 °C /min, 190–200 °C at 1 °C/min, 200–230 °C at 2 °C /min, 230–300 °C and held for 5 min. The temperature program on direct TMS derivatives was summarized as follows: 50 °C for 0 min, 50–190 °C at 40 °C /min, 190–200 °C at 0.5 °C/min, 200–210 °C at 1 °C /min, and 210–300 °C at 20 °C/min. The temperature procedure on alditol acetates was applied as follows: 140 °C for 0 min, 140–250 °C at 5 °C /min, and 250–300 °C at 10 °C /min. The temperature scheme on TMSO was described as follows: 150 °C for 0 min, 150–230 °C at 3 °C /min, and 230–300 °C at 20 °C/min. The temperature gradient on TMS of dithioacetals was employed as follows: 80 °C for 0 min, 80–190 °C at 2.5 °C/min, 190–252 °C at 2 °C/min, 252–300 °C at 25 °C/min, 300–310 °C and held for 15 min. Ionisation was performed in the electron impact mode at 70 eV. The ion source temperature was 230 °C, and the interface temperature was 250 °C. EI-spectra were recorded in full scan modus at *m/z* 50-750.

2.6. Fingerprint profiling and similarity evaluation

GC–MS chromatogram data of all samples were submitted for analysis by the professional software named "Similarity Evaluation System for Chromatographic Fingerprint of traditional Chinese medicine (SESCF-TCM)" published by China Pharmacopoeia Committee (Version 2004A and 2004B). SESCF-TCM was designed specifically for similarity evaluation of LC and GC fingerprints and has been recommended by the State Food and Drug Administration of China (Dou, Liu, Jiang, Zhang, & Liu, 2009; Ruan & Li, 2007). Then, the peaks would be matched and the reference (R) chromatogram would be produced. Subsequently, the correlation coefficient (c_r) value of all input chromatograms relative to standard chromatogram would be calculated by using the cosine value of the angle (Sun, Yan, Hou, Li, & Wang, 2015).