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One-pot preparation of a mixed-mode organic-silica hybrid monolithic capillary column and its application in determination of endogenous gibberellins in plant tissues

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ABSTRACT

A newly improved one-pot method, based on "thiol-ene" click chemistry and sol–gel approach in microemulsion system, was developed for the preparation of C_8/$PO(OH)_2$-silica hybrid monolithic capillary column. The prepared monolith possesses large specific surface area, narrow mesopore size distribution and high column efficiency. The monolithic column was demonstrated to have cation exchange/reversed-phase (CX/RP) mixed-mode retention for analytes on nano-liquid chromatography (nano-LC). On the basis of the developed nano-LC system with MS detector coupled to pipette tip solid phase extraction (PT-SPE) and derivatization process, we then realized simultaneous determination of 10 gibberellins (GAs) with low limits of detection (LODs, 0.003–0.025 ng/mL). Furthermore, 6 endogenous GAs in only 5 mg rice leaves (fresh weight) were successfully detected and quantified. The developed PT-SPE-nano-LC–MS strategy may offer promising applications in the determination of low abundant bioactive molecules from complex matrix.

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1. Introduction

Organic-silica hybrid monolithic stationary phases have been widely applied in capillary liquid chromatography (*c*LC) and capillary electrochromatography (CEC) due to their unique properties, such as fast mass transfer, good permeability and high column efficiency [1]. On the other hand, mixed-mode stationary phases have recently attracted extensive attention in HPLC analysis of small molecules. Nowadays, mixed-mode packed columns have been commercial available and widely used, and better separation can be achieved compared to single-mode packed columns [2–4]. However, the reported methods for the preparation of mixed-mode organic-silica hybrid monolithic capillary column often need fussy multistep. Though some one-pot strategies were recently developed [1,5,6], the organic-silica hybrid monolithic capillary column with strong hydrophobicity could not be prepared through these reported methods due to the incompatibility of hydrophobic monomer in sol–gel system. Recently, Lin et al. developed a one-pot method for preparing organic-silica hybrid monolithic capillary column with glutathione as functional group [7]. However, the application is limited due to less monomer. Therefore, significant challenges remain in the development of facile and universal methods for preparation of mixed-mode organic-silica hybrid monolithic capillary column.

Gibberellins (GAs), a class of plant growth hormones, play essential roles in the regulation of plant growth and developmental processes, including stem elongation, germination, flowering and fruit development [8,9]. Natural GAs are a group of diterpenoid acids and exist in almost all green plants [10]. Determination of endogenous GAs is critical for the in-depth understanding of their biological functions in plants. However, effective detection of GAs is normally difficult due to the low abundance of endogenous GAs present in plants as well as the serious effect of plant matrix [11,12]. Therefore, development of sensitive and selective analytical methods for the determination of GAs is highly needed.

Liquid chromatography–mass spectrometry (LC–MS) has been demonstrated to be a powerful platform for sensitive determination of phytohormones [13–15]. However, the MS-based methods are still restricted to achieve satisfactory MS response for negatively charged carboxylated phytohormones such as GAs, mainly due to their low ionization efficiency [16], matrix effect [17], and co-elution of analytes [18]. In this respect, derivatization is an effective strategy and frequently used to improve the MS detection sensitivity of target compounds through labeling the compounds

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with easily ionized group [19–23]. For example, to enhance the ionization efficiency, a derivatization reagent (mass probe) with quaternary amine group was employed to label GAs [24]. However, the separation efficiencies of some GA derivatives, such as GA_4, GA_{51}, GA_1 and GA_3 are very poor on C_{18} column. Similarly, GA derivatives generated from other derivatization reagents with tertiary amine group also have the same problem [23,25]. Thus, effective separation of these GA derivatives in suitable separation mode is favorable.

In the meantime, although conventional particulate packed columns were widely employed for GAs analysis, a relatively large plant amount was normally required. Nano-LC was demonstrated to be an effective approach to reduce the sample amount and improve detection sensitivity [26]. Recently our group developed a nano-LC–MS platform by using porous polymer monolithic capillary column as the separation medium for the sensitive determination of phytohormones in plant tissues [24], and 5-methylcytosine and 5-hydroxymethylcytosine in genomic DNA [27]. In this respect, monolithic stationary phases are promising alternative for nano-LC due to the good permeability and broad selectivity. Considering for the chemical properties of mass probe-derived GAs and the advantages of monolithic stationary phase, a hydrophobic/cation exchange monolithic capillary column may provide good separation resolution for the target analytes.

In this current study, a newly improved one-pot method, based on "thiol-ene" click chemistry and sol–gel approach in microemulsion system, was developed for the preparation of C_8/$PO(OH)_2$-silica hybrid monolithic capillary column. The prepared monolith possesses large specific surface area, narrow mesopore size distribution and high column efficiency. The monolithic column was demonstrated to have cation exchange/reversed-phase (CX/RP) mixed-mode retention for analytes on nano-LC. To develop a method for determination of endogenous GAs in plant tissues on the basis of nano-LC–MS with CX/RP monolithic capillary column, we also proposed a sample preparation strategy combining pipette tip solid phase extraction (PT-SPE) and derivatization process. Taken together, we achieved the simultaneous determination of 10 GAs with low limits of detection (LODs, 0.004–0.032 ng/mL) using PT-SPE-nano-LC–MS system. Furthermore, the developed method was successfully applied to the determination of endogenous GAs in only 5 mg rice leaves (fresh weight).

2. Materials and methods

2.1. Chemicals

2,2-Azobisisobutyronitrile (AIBN), urea (>95%, w/w), sodium dodecyl sulfonate (SDS, >95%, w/w), ammonia water (containing 25–28% NH_3, w/w), and poly (ethylene glycol) with the molecular weight of 10,000 (PEG-10000) were all purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). AIBN was purified by recrystallization from ethanol at 40 °C. Tetramethoxysilane (TMOS, 98%, w/w), n-octyltrimethoxysilane (C_8-TMOS, 98%, w/w), octadecyltrimethoxysilane (C_{18}-TMOS, 98%, w/w) and γ-mercaptopropyltrimethoxysilane (SH-TMOS, 98%, w/w) were purchased from Wuhan University Silicone New Material (Wuhan, China). The fused-silica capillaries (75 μm i.d., 360 μm o.d.) were purchased from Yongnian Optic Fiber Plant (Hebei, China). Stable isotope-labeled compounds and standards, [$^2H_2$] GA_1 (internal tracers of GA_1 and GA_3), [$^2H_2$] GA_4 (internal tracers of GA_4 and GA_7), [$^2H_2$] GA_9 (internal tracers of GA_9), [$^2H_2$] GA_{19} (internal tracers of GA_{19} and GA_{44}), [$^2H_2$] GA_{51} (internal tracers of GA_5 and GA_{51}), [$^2H_2$] GA_{53} (internal tracers of GA_{53}), GA_1, GA_3, GA_4, GA_5, GA_7, GA_9, GA_{19}, GA_{44}, GA_{51}, GA_{53}, Zeatin-9-glucoside (Z9G), zeatin-riboside (ZR), N^6-isopentenyladenine-9-glucoside (iP9G), isopenteny-ladenine riboside (iPR), and zeatin (Z) were purchased from Olchemim Ltd. (Olomouc, Czech Republic). The structure of GAs can be seen in Fig. S1. Thiourea (>95%, w/w), toluene, ethylbenzene (>95%, w/w), propylbenzene (>95%, w/w), ammonium formate ($HCOONH_4$, 98%, w/w), formic acid (FA, >88%, w/w), and triethylamine (TEA, 98%, w/w) were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). 2-Chloro-1-methyl-pyridinium iodide (CMPI, 98%, w/w) and *N*,*N*-diethyl ethylenediamine (DEED) (98%, w/w) were purchased from Aladdin (Shanghai,China). HPLC-grade acetonitrile (ACN) were obtained from TEDIA Company Inc. (OH, USA). Milli-Q water (Millipore, Bradford, USA) was used in all experiments. HiCapt SAX SPE adsorbent, a strong anion exchanger containing quaternary ammonium group, was purchased from Weltech Company (Wuhan, China). The particle size was labeled as 200–300 mesh. The pipette tips for PT-SPE were prepared by packing the SAX adsorbent into a 200-μL pipette tip, with a polyethylene frit for blocking the SAX sorbent. The frits (approximately 1.0 mm diameter, 1.4 mm thickness) were purchased from Biocomma (Shenzhen, China). All other reagents were of analytical reagent grade unless otherwise indicated.

2.2. Preparation of the monolithic capillary columns

The fused-silica capillaries were washed with 1 mol/L NaOH (2 h), H_2O (30 min), 1 mol/L HCl (1 h), H_2O (30 min), and methanol (30 min) successively to activate the silanol groups. Then the capillaries were allowed to dry under nitrogen flow at 160 °C for 5 h.

C_8/$PO(OH)_2$-silica hybrid monolithic capillary column was prepared using improved one-pot approach. Typically, H_2O (500 mg, 61.1% w/w total), PEG-10000 (90 mg, 11.0% w/w total), ammonium hydroxide (7.5 mg, 0.9% w/w total) and vinylphosphonic acid (10 mg, 1.2% w/w total) were mixed to form solution A. TMOS (185 mg, 22.6% w/w total), SH-TMOS (10 mg, 1.2% w/w total), n-octyltrimethoxysilane (15 mg, 1.8% w/w total), SDS (1 mg, 0.1% w/w total) and AIBN (1 mg, 10% w/w SH-TMOS) was mixed to form solution B. Subsequently, 212 mg of solution B was added to 607 mg of solution A and degassed by a 10-min ultrasonication. The homogeneous mixture was then manually introduced into the fused-silica capillary to an appropriate length by a syringe. After both ends of the capillary were sealed with two pieces of rubber, the mixture was incubated at 40 °C for 12 h for simultaneous polycondensation and "thiol-ene" click reaction. The resulting monolith was completely flushed with water and ACN successively to remove the PEG and other residuals.

For comparison, a $PO(OH)_2$-silica hybrid monolithic capillary column (30 cm-long, 75 μm i.d., 360 μm o.d.) was also prepared. The preparation procedure is the same as that for the preparation of C_8/$PO(OH)_2$-silica hybrid monolithic capillary column but without the addition of C_8-TMOS.

C_{18}-silica monolithic capillary column (30 cm-long, 75 μm i.d., 360 μm o.d.) used for control experiment was prepared according to previous report [28]. Detail information can be seen in Supporting Information.

2.3. Characterization of the monolithic capillary columns

The surface area and pore size distribution were measured by a specific surface area and pore size distribution analyzer (Beijing JWGB Sci. & Tech., Beijing, China). The microscopic morphology was examined by scanning electron microscope (SEM), using a Quanta 200 scanning electron microscope (FEI Company, Holland). The elemental contents of the prepared monoliths were determined on Shimadzu EDX-720 energy-dispersive X-ray analysis (EDX, Kyoto, Japan) by using Mg–Ka radiation as the excitation source. Permeability measurements were performed by using a Shimadzu

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