Simultaneous Determination of *p*-Hydroxybenzaldehyde, *p*-Hydroxybenzyl Alcohol, 4-(β-D-Glucopyranosyloxy)-Benzyl Alcohol, and Sugars in *Gastrodia elata Blume* Measured as Their Acetylated Derivatives by GC–MS

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Abstract

A method for the simultaneous separation and determination of the active constituents and three sugars in the roots of *Gastrodia elata Blume* (GE), which is used as a famous Chinese traditional herbal medicine, by gas chromatography–mass spectrometry is established. The samples are acetylated with pyridine–acetic anhydride. The contents of 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol (HA), fructose, glucose, 4-(β-D-glucopyranosyloxy)-benzyl alcohol (GA), and sucrose in GE are 0.004%, 0.03%, 1.36%, 1.12%, 1.97%, and 4.25%, respectively, and the detection limits are 1.5, 3.0, 11.0, 5.0, 33.0, and 35.0 pg, respectively. The contents of HA and GA in the urine and brain of a mouse are also determined. This method is simple, reliable, and quick for the simultaneous determination of the active constituents and sugars in GE.

Introduction

Gastrodia elata Blume (GE) is a traditional Chinese herbal medicine that has been used for many years for the treatment of convulsions and epilepsy. The extracts of its rhizome promote blood circulation and cure headaches. It is prescribed for rheumatism, neuralgia, paralysis, lumbago, and other neuralgic and nervous affections (1,2). Chemical studies have shown that GE rhizome contains mainly 4-hydroxybenzyl alcohol (HA), 4-(β-D-glucopyranosyloxy)-benzyl alcohol (GA), 4-hydroxybenzaldehyde (HD), sucrose, β-sitosterol, and citric acid. HA and GA have been reported as the main active constituents of this plant (3–5). Several methods for the determination of GA or HA have been reported such as spectrophotometry, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) (6,7), and capillary electrophoresis (CE) (8). However, most of these methods seem to be unsuitable for the quantitative determination of these compounds because of either poor resolution or low sensitivity. In this study, we first developed a gas chromatography (GC)–mass spectrometry (MS) method to determine GA, HA, HD, and sugars. The quantitation of sugars in GE has not been reported so far. It is important to determine the sugars as well because sugars are the main constituents of GE and are involved in very important characteristics such as maturity, ripeness, quality, authenticity, and storage conditions. Sugars are often determined as their trimethylsilyl-oxime ethers or esters or both by GC (9). This method has shortcomings because water reacts with reagents. Sawardeker et al. (10) developed a derivatization scheme in which the sugars were reduced with sodium borohydride, then the solution was evaporated to dryness and the dehydrated residue was acetylated by 1:1 acetic anhydride–pyridine overnight. In a previous study (3), GA was successfully acetylated directly with 1:1 acetic anhydride–pyridine below a 90°C water bath for 2 h. In this study, sugars together with HA, GA, and HD were directly acetylated employing the previously mentioned method. Each monosaccaride (glucose and fructose) produced two acetylated anomers, which were baseline separated. Sucrose, HA, HD, and GA produced a single acetylated compound, respectively. This derivatization provided good reproducibility. It was the first time that sugars, HA, HD, and GA were simultaneously acetylated directly with acetic anhydride–pyridine without reduction reaction. The main constituents in GE can be separated and determined as their acetylated derivatives from one solution by one injection within 22 min. They can also be separated completely from their neighbors evaluated by means of their extract ion chromatogram (EIC) at the low picogram concentration level. This was the first time a GC–MS method was developed to simultaneously separate and identify the main constituents (GA, HA, HD, sucrose, glucose, and fructose) of GE. We also analyzed mice brain extract and urine after the oral administration of GE extracts by GC–MS, which was much simpler and quicker than the radioactivity method (11).

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Experimental

Reagents

HA and HD of analytical grade were purchased from Sigma (St. Louis, MO). GA of analytical grade was purchased from Chinese Medicine Control Institute (Beijing, China). Sucrose, glucose, and fructose of analytical grade were purchased from Beijing Chemical Reagents Company (Beijing, China). The methanol used was of HPLC grade and other chemicals were of analytical grade.

The crude medicinal material of GE was purchased from the Sichuan province of China (Chengdu, Sichuan, China).

Conditions of GC–MS

The GC–MS system consisted of an HP6890 GC, an HP5973 mass-selective detector, and an HP ChemStation data-analysis system. The running conditions were as follows. Separation was performed on an HP-5 MS capillary column (30.0 m × 250 µm × 0.25 µm, 5% phenyl methyl siloxane). The temperature program began at 50°C, rose to 200°C at 20°C/min, rose to 220°C at 5°C/min, rose to 280°C at 10°C/min, and then was held at 280°C for 5 min. The injector temperature was 280°C, and the MS source temperature was 250°C. The electron multiplier voltage was 70 eV. The flow rate of the carrier gas (He) was 0.8 mL/min with a split ratio of 30:1. A full-scan acquisition mode (*m/z* 35–500) was used for detection, and the sample size was 1 µL.

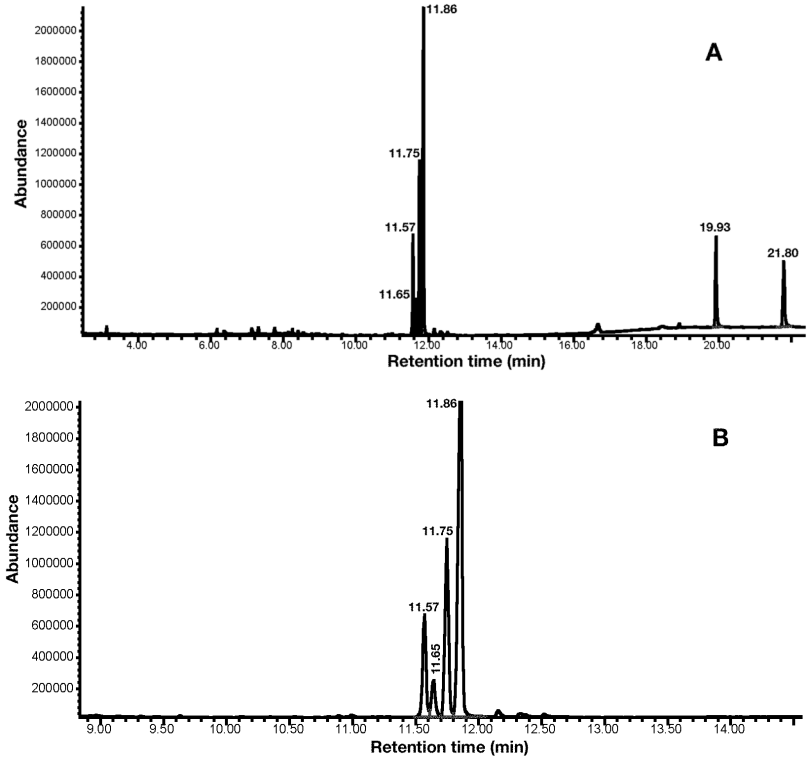

Figure 1. GC–MS chromatograms of the GE extract: (A) total ion chromatogram and (B) local magnification of the total ion chromatogram.

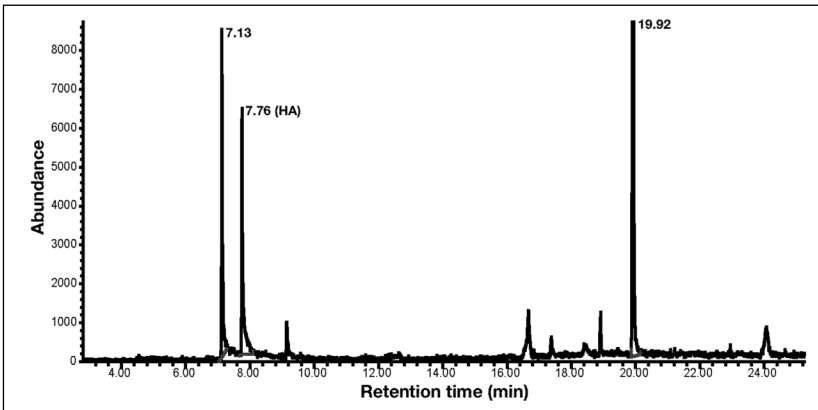

Figure 2. GC–MS chromatogram of the GE extract analyzed by the extract ion at *m/z* 107.

Preparation of the GE extract

Ten grams of GE rhizome was ground and extracted with 100 mL aqueous ethanol (80% ethanol) by refluxing on a water bath at 80°C for 1 h. Then, it was placed in an ultrasonic bath for 10 min. Extraction was repeated three times. The extracts were filtered with Whatman No. 1 filter paper. The filtrate was evaporated at 50°C with a vacuum rotary evaporator, and the extract was dried with nitrogen gas. The yield of extract was 12%. The extract was acetylated with pyridine–acetic anhydride for GC–MS analysis.

Derivatization of the extract and standards

GA was acetylated with a 1:1 mixture of acetic anhydride and pyridine for 2 h at 90°C [3]. The product was evaporated to dryness by nitrogen, and the residue was dissolved in methanol for a chromatographic analysis. The same method was used for the derivatization of other standards and the GE extract.

Treatment of the biological specimens

GA and HA were assayed in brain and urine samples of a mouse after an oral administration of GE extract.

Brain

The whole brain of a mouse was weighed, the blood was washed off, and then the brain was ground into slurry along with 5 mL physiological saline solution. After centrifugation, the supernatant was added to three times the volume of methanol in order to precipitate proteins. The clear solution was evaporated to dryness by nitrogen, and then the residue was derivatized with pyridine–acetic anhydride as described.

Urine

Two times the volume of ether was added in the urine, then the solution was evaporated to dryness under air current. The residue was derivatized with pyridine–acetic anhydride as described.

Results and Discussion

Separation and identification of HA, GA, HD, sucrose, glucose, and fructose in GE

The total ion chromatograms of the GE derivatives are shown in Figure 1. The constituents were baseline separated within 22 min. Compared with the retention time and the mass spectra of standard acetylated derivatives, it can be determined that the peak with a retention time of 21.80 min was the acetylated sucrose derivative, the peak of 19.92 min was the GA derivative, the peaks of 11.57 min and 11.65 min were two anomers of acetylated fructose, and the peaks of 11.75 min and 11.86 min were two anomers of acetylated glucose, which were inferred from the chromatograms of the acetylated standard fructose and glucose derivatives. The peaks of 7.76 min and 6.47 min corresponding to the acetylated HA and acetylated HD, respectively, were not visible in Figure 1 because of their low contents compared with those of sugars in the GE extract. When using EIC to analyze Figure 1, the selectivity and the signal-to-noise ratio improves greatly. The EIC (*m/z* = 107, Figure 2) displayed the presence of acetylated HA with a retention time of 7.76 min. Acetylated HD was identified using the same method (extract ion *m/z* = 121). The extract ions, which were the characteristic ions with greater intensity displayed in the mass spectrum patterns of each compound, are shown in Table I.

Table I. Characteristic Data of Acetylated Compounds in GE

Compound	MW of Acetylated compound	Retention time (min)	Extract ion	Other important ions
HD	164	6.47	121	164,65,105,136
HA	208	7.75	107	166,124,78, 207,152
Fructose	390	11.57,11.65	101	113,145,169, 186,211,275, 228,331
Glucose	390	11.75,11.86	115	98,157,140, 169,200,242, 317,331,347
GA	496	19.93	169	43,109,127, 139,331
Sucrose	678	21.80	169	109,211,331, 127,271,139, 157,229

Further identification was confirmed by comparing the mass spectra of the GE extracts with those of authentic compounds. The mass spectrum patterns of the sample were similar to those of the authentic (figure not shown). The main fragment ions of each compound are summarized in Table I. When comparing the retention time and the mass spectra of the standard and GE extract, the main compounds of the GE extract with retention times at 6.47, 7.76, 11.57, 11.65, 11.75, 11.86, 19.93, and 21.80 min can be identified as acetylated derivatives of HD, HA, fructose, fructose, glucose, glucose, GA, and sucrose, respectively.

The peak with a retention time at 7.13 min (Figure 2) showed prominent peaks at *m/z* (relative intensity) 152 (M+, 36), 107 (100), 123 (10), and 77 (13). Because no corresponding standard was available, the further identification of the compound was not performed.

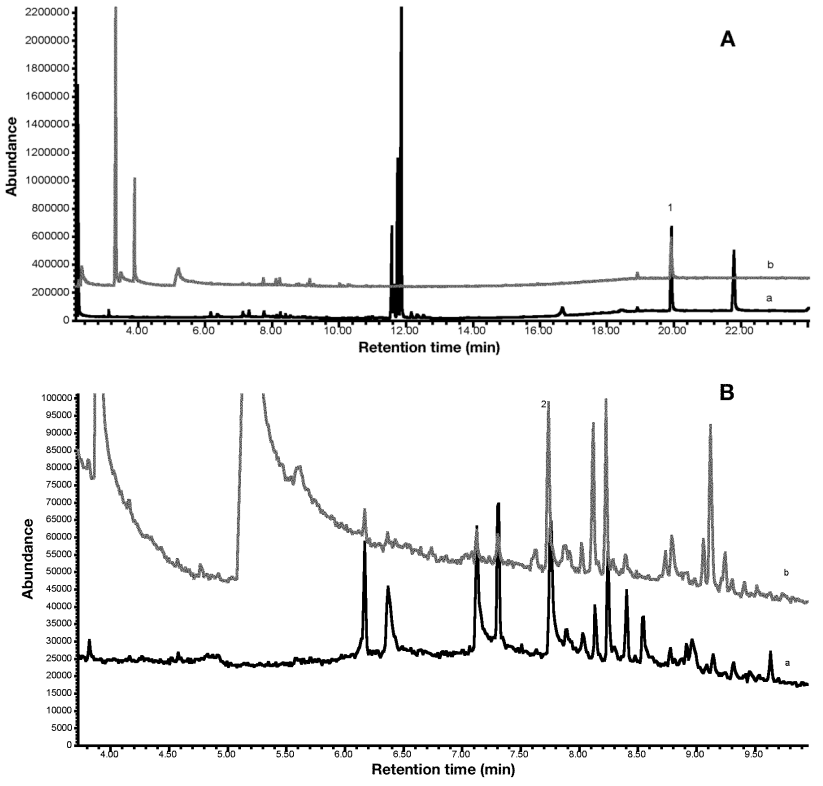

Figure 3. Comparison of GC–MS chromatograms of (a) herbs and (b) urine: (A) total ion chromatogram, (B) local magnification, (1) GA, and (2) HA.

Identification of HA and GA in mice urine and brain

Because HA and GA are the main active constituents of GE rhizome, the determination of HA and GA in mice urine and brain after oral administration of GE extracts was performed.

When comparing the chromatograms of urine and GE (Figure 3), it can be seen that the two graphs have the same peaks at 7.76 min and 19.92 min, which were correspondent to HA and GA derivatives, respectively. Further identification was confirmed by comparing the mass spectra with those of standards.

The constituents of the mouse brain were complicated. From the EICs (*m/z* 107 for HA and *m/z* 109 for GA, figure not shown), HA and GA can be detected, which showed peaks at 7.73 and 19.91 min, respectively. The relative contents of HA and GA were different from that of the GE extract, probably because GA decomposed into HA in the brain [12]. The results demonstrated that GA could penetrate through the blood–brain barrier into the brain.

Table II. Components of GE Extract and Mice Urine and Brain Samples Determined as Their Acetylated Derivatives on the Base of Their Fragment Ions

	HD	HA	Fructose	Glucose	GA	Sucrose
GE extract (g/100 g)	0.004	0.03	1.36	1.12	1.97	4.25
Urine (μg/mL)		1.83			212	
Brain (μg/g)		0.15			0.78	

Quantitation of the main constituents of GE extracts and HA and GA in specimens

The contents of compounds were calculated by the external standard method. Quantitation was performed by EIC using mass-to-charge ratios of 169 for GA and sucrose, 107 for HA, 115 for glucose, 101 for fructose, and 121 for HD. The selectivity was improved greatly. The results are shown in Table II. Chromatographic precision, expressed as relative standard deviation (RSD), was calculated by injecting five replicates of the GE extract. RSD varied between 1.7% and 3.4%. The detection limits (signal-to-noise ratio = 3) of HD, HA, fructose, glucose, GA, and sucrose were 1.5, 3, 11, 5, 33, and 35 pg, respectively.

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