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LC/TIS-MS Fingerprint Profiling of *Cimicifuga* Species and Analysis of 23-Epi-26-deoxyactein in *Cimicifuga racemosa* Commercial Products

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In this study, a liquid chromatography (LC)/turbo ion spray (TIS)−mass spectrometry (MS) method was developed to examine the chromatography or "fingerprint profile" of seven *Cimicifuga* herbs and six *Cimicifuga racemosa* (black cohosh) commercial products. Triterpene glycoside components were selected as chemical markers for analysis because they have appeared as a major compound group in *Cimicifuga* species. LC/MS chromatograms unveiled the patterns of *C. racemosa*, *Cimicifuga dahurica*, *Cimicifuga foetida*, *Cimicifuga heracleifolia*, *Cimicifuga japonica*, *Cimicifuga acerina*, and *Cimicifuga simplex*, which are very different from each other. 23-Epi-26-deoxyactein was found only in *C. racemosa*, *C. dahurica*, and *C. foetida*. A highly selective and sensitive LC/MS/MS method for quantitative analysis of 23-epi-26-deoxyactein with detection levels up to 2.5 ng in these samples was also developed and was applied to six commercial *C. racemosa* products. *C. racemosa* and its six commercial products contained about 6−15% of 23-epi-26-deoxyactein in total triterpene glycosides. On the other hand, the estimated amount of total triterpene glycosides in other commercial products was either greater or lesser than what the manufacturers claimed. The technique and LC/MS profiles generated in this study provide a reliable and reproducible method that can be readily utilized for botanical identification of *Cimicifuga* plants, for examination and validation of its commercial products, and for "chemical" quality control in the manufacture of black cohosh products.

KEYWORDS: ***Cimicifuga*; black cohosh; Ranunculaceae; 23-epi-26-deoxyactein; LC/MS**

INTRODUCTION

The roots and rhizomes of *Cimicifuga* species are important medicinal herbs. There are about 10 species of *Cimicifuga* genus plants existing in the North Temperate Zone (*1*). In China, *Cimicifuga dahurica* (Turcz.) Maxim., *Cimicifuga foetida* L., and *Cimicifuga heracleifolia* Kom. and, in Japan, *Cimicifuga simplex* Wormskjord., *Cimicifuga japonica* (Thunb.) Sprengel, and *Cimicifuga acerina* (Sieb. et Zucc.) C. Tanaka have historically been used as botanical sources for important traditional herbal drugs called "Sheng Ma". Similarly, *Cimicifuga racemosa* (L.) Nutt. (black cohosh) has also been widely used in the United States and Europe.

C. racemosa is a shrublike plant native to the eastern deciduous forests of North America, ranging from southern Ontario to Georgia, north to Wisconsin, and west to Arkansas. Recently, it has been reclassified into the genus *Actaea* by DNA evidence (*2*). The dried roots and rhizomes of *C. racemosa* have been used by Native Americans for many conditions, ranging from gynecological problems to rattlesnake bites. Some 19th century American physicians used *C. racemosa* for fever, menstrual cramps, arthritis, and insomnia (*3*). Many *C. racemosa*-based diet supplements are currently sold in the United States and Europe for alleviation of menopausal and postmenopausal symptoms such as hot flashes. Clinical trials of *C. racemosa* are ongoing in the United States, and one report showed that black cohosh extract was ineffective in treating hot flashes in women with a history of breast cancer (*4*).

C. racemosa contains many different compounds (*1*, *3*, *5*−*8*), including triterpene glycosides such as 23-epi-26-deoxyactein (formerly 27-deoxyactein) (*5*) and cimicifugoside H-1 (**Figure 1**), aromatic acids, and isoflavones. Although there are many commercially available *C. racemosa* products, the quality of these products is questionable due to the lack of a well-recognized standard and quality control. Many products claim to contain 1−2 mg per serving of triterpene glycosides calculated as 23-epi-26-deoxyactein, but this claim has not been proven. Triterpene glycosides of *C. racemosa* have a weak UV absorbance, which makes them difficult to analyze by conventional

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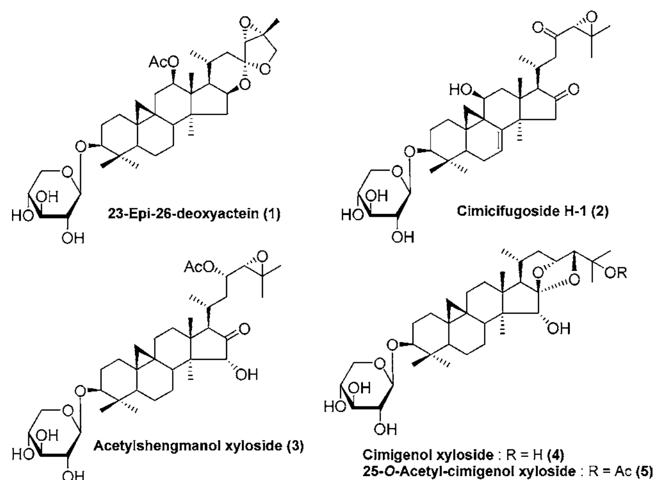

Figure 1. Structures of some *Cimicifuga* triterpene glycosides.

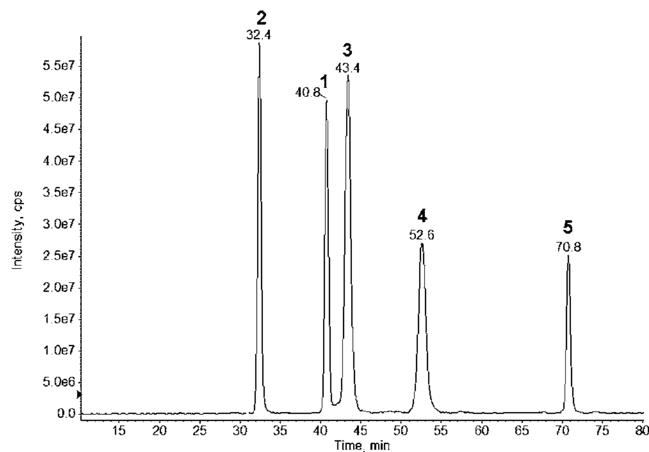

Figure 2. Typical TIS-MS HPLC profile of five standard compounds from *Cimicifuga*. Compounds: 1, 23-epi-26-deoxyactein; 2, cimicifugoside H-1; 3, acetylshengmanol xyloside; 4, cimigenol xyloside; and 5, 25-*O*-acetylcimigenol xyloside.

UV−high-performance liquid chromatography (HPLC) methods. Recently, several analytical methods have been developed to analyze the constituents of *C. racemosa.* Li (*9*) and Ganzera (*10*) have employed evaporative light scattering detection (ELSD)/HPLC to detect the triterpene glycosides and aromatic acid esters. Atmospheric pressure chemical ionization (APCI)-LC/MS for triterpene glycosides (*11*), electrospray ionization/tandem mass spectrometry (LC/MS/MS) for caffeic acid derivatives (*12*), and LC/MS and immunoaffinity extraction for evaluation of triterpene glycosides estrogenic activity (*13*) have been reported. A validated quantitative method for 16 phenolic and triterpenoidal compounds has also been reported (*9*).

In this study, we developed a LC/turbo ion spray (TIS)-MS method to examine the LC/MS chromatography or "fingerprint profile" of *Cimicifuga* herbs and *C. racemosa* products. Triterpene glycosides components were first selected because they have appeared as a major compound group in *C. racemosa*. A highly selective and sensitive LC/MS/MS method for analyzing a chemical marker (23-epi-26-deoxyactein) was also developed. The methods developed provide a tool for the rapid identification of *Cimicifuga* plants and quality control for the manufacture of *C. racemosa* products.

MATERIALS AND METHODS

Solvents and Reagents. Chloroform, methanol, and HPLC grade ammonium acetate were purchased from Fisher Scientific (Atlanta, GA). HPLC grade acetonitrile (CH_3CN) and methanol (CH_3OH) were from Aldrich (St. Louis, MO). Deionized water was purified from distillated water by an EASYpure RF water purifier (Dubuque, IA).

Plant Material and *C. racemosa* Commercial Products. *C. racemosa* (black cohosh) was purchased from Leaves and Roots (Florida). *C. dahurica*, *C. foetida, and C. heracleifolia* were collected from China in 2001 and were identified by Professor Zheng-Tao Wang at the Shanghai University of Traditional Chinese Medicine (Shanghai, China) and Professor Dao-Feng Chen at School of Pharmacy, Fudan University (Shanghai, China). *C. simplex*, *C. japonica*, and *C. acerina* were collected from Japan in 2002 and were identified by Dr. Seiji Nagumo at Hoshi University (Tokyo, Japan). Voucher samples were deposited in AndroScience's herbarium. The other six *C. racemosa* commercial products covering different dosage forms were purchased from stores in North Carolina. 23-Epi-26-deoxyactein and other triterpene glycosides used as authentic compounds were isolated and purified from *Cimicifuga* species by procedures mentioned in the literature (*1*, *8*), and their structures were confirmed by various spectroscopies.

Standards and Samples Preparation. The herb materials or commercial products were ground with a small mill to powder with a size less than 16 mesh. The powder was dried in a desiccator for 12 h at room temperature. One gram of powder was weighed and was put into 50 mL flasks. The powder was extracted by 10 mL of chloroform−methanol (1:1, v/v) with stirring for 1 h and repeated three times. All extracts was combined, and the solvent was removed by vacuum evaporation at 50 °C. The residue was dried in a vacuum chamber at room temperature for 2 h. HPLC grade methanol was added to the residue to make a solution with a concentration of 1.0 mg/mL. The solution was filtered by a Whatman Puredisk 25 TF filter to remove small particles or any insoluble substances.

Instruments. HPLC analyses were carried out on an Agilent 1100 HPLC system equipped with a binary pump, a four-channel online degasser, a thermostatic autosampler, a column oven, and an UV/vis detector. Mass spectra were recorded on a PE-Sciex API-3000 LC/MS/MS instrument equipped with a TIS ion source. Nitrogen gas was used as the nebulizing and curtain gas at a pressure of 12 psi. The TIS potential was set at 3.5 kV. A mass range of 400−1000 amu was scanned at a scan time of 1 s. Eluate from the column was split between the UV detector and the mass instrument at a ratio of 4:1. The PE-Sciex Analyst Version 1.1 was used for instrument controls and data processing.

HPLC Solvent Gradient Profile. HPLC conditions included the use of a 150 mm × 3.0 mm i.d., 4 μm, Phenomenex Synergi Hydro-RP 80A C_{18} with polar end-capping reverse phase column at 20 °C with a flow rate of 1.0 mL/min. The solvent system consisted of acetonitrile (solvent A) and 10 mM ammonium acetate in deionized water (solvent B). The gradient profile for LC/MS fingerprint profiles was as follows: analytical cycle (0−18 min, 5−28% A; 18−35 min, 28−35% A; 35−50 min, 35% A; 50−70 min, 35−55% A; and 70−80 min, 55−75% A) and wash and restore column cycle (80−90 min, 75−100% A; 90−93 min, 100% A; 93−95 min, 100 to 5% A; and 95−97 min, 5% A). The gradient profile for quantitative analysis was as follows: analytical cycle (0−18 min, 5−28% A; 18−35 min, 28−35% A; and 35−45 min, 35% A) and wash and restore column cycle (45−47 min, 35−100% A; 47−50 min, 100% A; 50−53 min, 100 to 5% A; and 53−55 min, 5% A). An injection volume of 50 μL (50 μg on column) was used.

Detection Limits and Linearity. The lower limit of quantitation of 23-epi-26-deoxyactein was determined to be 2.5 ng, and the upper limit of quantitation was determined to be 5000 μg/mL without ion suppression. A series of standard solutions (500, 250, 125, 62.5, 31.3, 15.6, 7.8, and 3.9 μg/mL) of 23-epi-26-deoxyactein, which were within the range of lower and upper limits of quantitation of 23-epi-26-deoxyactein, were prepared in acetonitrile. A calibration curve of 23-epi-26-deoxyactein with a regression value of 0.997 was obtained.

RESULTS AND DISCUSSION

LC/MS Profiles of Standard Compounds Isolated from *Cimicifuga*. Five standard compounds isolated from *Cimicifuga*

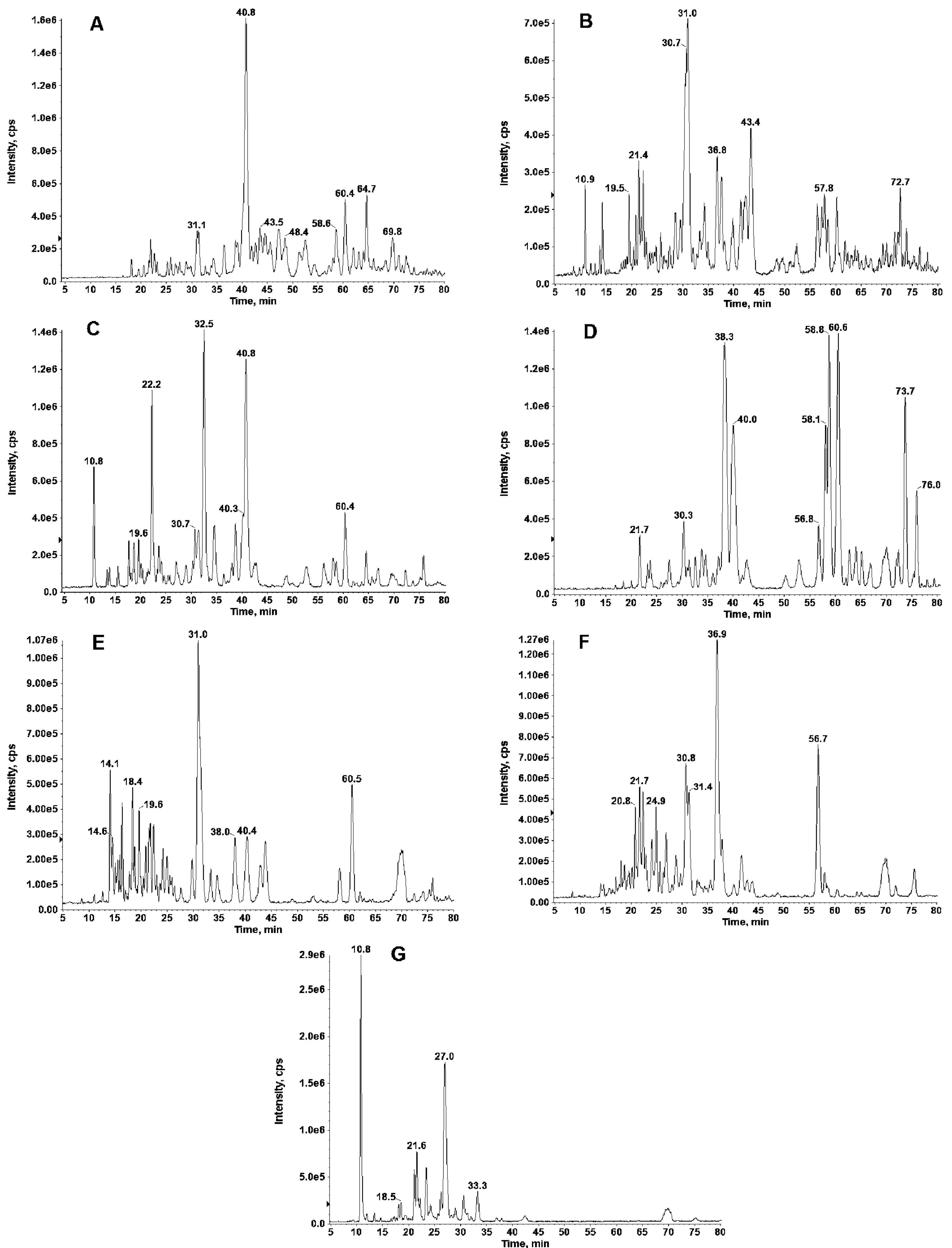

Figure 3. Base peak ion chromatograms of seven *Cimicifuga* plants. (**A**) *C. racemosa*, (**B**) *C. dahurica*, (**C**) *C. foetida*, (**D**) *C. heracleifolia*, (**E**) *C. japonica*, (**F**) *C. acerina*, and (**G**) *C. simplex*.

Table 1. Characteristic Peaks and Structural Information of Seven *Cimicifuga* Plants in **Figure 2**

plant name	retention times, min[a] (molecular weight[b])
C. racemosa	40.8 (660), 64.7 (528), 60.4 (620), 43.5 (662), 47.2 (620), 58.6 (620), 31.1 (766[c]), 48.4 (662), 69.8 (662), 52.6 (620)
C. dahurica	31.0 (766), 43.4 (662), 36.8 (764), 21.4 (766), 22.3 (766), 37.6 (766), 10.9 (468), 72.7 (746), 57.8 (662), 43.4 (662)
C. foetida	32.5 (616), 40.8 (660), 22.2 (632), 10.8 (468), 60.4 (602), 38.7 (658), 34.6 (706), 30.7 (500), 31.4 (766), 19.6 (722)
C. heracleifolia	60.6 (620), 58.8 (620), 38.3 (704), 73.7 (486), 40.0 (706), 58.1 (662), 76.0 (488), 30.3 (722), 56.8 (660), 21.7 (720)
C. japonica	31.0 (748), 14.1 (738), 18.4 (722), 60.5 (620), 16.4 (652), 19.6 (722), 21.7 (722), 21.5 (766), 38.0 (766), 40.4 (706)
C. acerina	36.9 (764), 56.7 (660), 30.8 (764), 21.7 (722), 22.3 (834), 31.4 (766), 20.8 (854), 24.9 (896), 26.9 (850), 24.1 (834)
C. simplex	10.8 (468), 27.0 (836), 21.6 (894), 21.1 (896), 33.3 (850), 26.3 (880), 30.6 (878), 18.5 (854), 22.2 (912), 18.1 (852)

[a] Retention times are in descending order by their peak heights on the base peak chromatograms. [b] Determined by its $[M + NH_4]^+$ and/or $[M + H]^+$ adduct ions. [c] Molecular weights with underline marks are not reported in the literature so far.

species were used to determine their retention times and to confirm separation efficiency. **Figure 2** shows the LC/MS profile of these compounds. All compounds showed fairly symmetric and sharp peaks using the HPLC conditions aforementioned. Five triterpene glycosides were well-separated despite the similarity of their structures. It is interesting that compounds **2**, **3**, and **5** have large differences in retention times although they possess the same number of hydroxyl groups. Using TIS as the softest ionization method, these triterpene glycosides showed only molecular ions and adduct ions as dominant and base peaks without significant fragmentation.

LC/MS Fingerprint Profiles of *Cimicifuga* Species. Figure 3 shows the TIS + LC/MS profiles of seven *Cimicifuga* species. These regenerated base peak chromatograms (BPC) are almost identical with their original total ion chromatograms but show flatter baselines and greater peak separations. Many well-separated peaks could easily be recognized, and the profiles of all species are different from each other. **Table 1** lists the 10 most characteristic peaks and the molecular weight of each peak based on the BPC of each samples. It is noticeable that most characteristic peaks of *C. racemosa* and about half of them of three Chinese species were identifiable as known compounds. However, molecular weights of the most characteristic peaks of three Japanese species are over 700 and were unidentifiable as known compounds. To further unveil more structural information from LC/MS data, extract ion chromatograms (XIC) were used to identify peaks as known compounds, which have been reported in the literature, and the results were summarized in **Table 2**. The data presented in **Table 2** are intended to provide useful information for further studies rather than to replace full identifications by standard references. Because many isomers of triterpene glycosides exist in *Cimicifuga* herbs, an unambiguous identification needs a standard to check both retention time and molecular weight. Five standards (**Figure 2**) were used in this study, and all of them were identified from different species (**Table 2**). For many other compounds, although there was a lack of their standards, they were still identifiable with quite confidence by their unique molecular weight and exclusive distribution in the species. However, some compounds were still undistinguishable from multiple retention times and some were undetected despite the fact that they have been reported in the literature. In addition, some compounds (with √ sign in **Table 2**) were identified by cross-reference to other species although their existences in the corresponding species were not reported. The most intense peak at a retention time of 40.8 min in *C. racemosa* (**Figure 3A**) was unambiguously identified as 23-epi-26-deoxyacteinthree along with other three compounds (acetylshengmanol xyloside, cimigenol xyloside, and 25-acetylcimigenol xyloside) by their mass spectra and retention times as compared with standards. In addition, more than 20 other compounds were also detected with or without retention times identified. 23-Epi-26-deoxyactein was also observed as a distinguished peak in *C. foetida* (**Figure 3C**) but not in the other five species. *C. dahurica* (**Figure 3B**) showed two unseparated peaks at about 30 min as major peaks with a mass of 764 and 766. *C. foetida* had more significant polar peaks than nonpolar peaks. The peak at 32.5 min was readily identified as cimicifugoside H-1 (*2*) along with 23-epi-26-deoxyactein by their standard references. In contrast, *C. heracleifolia* (**Figure 3D**) had more abundant nonpolar peaks than polar peaks. At least five well-separated peaks were observed between the time ranges of 50−80 min. All other three Japanese species, *C. japonica* (**Figure 3E**), *C. acerina* (**Figure 3F**), and *C. simplex* (**Figure 3G**), showed more of the polar components than the less polar ones. *C. japonica* demonstrated the largest peak at 31.0 min as well as many well-separated peaks in the range of 14−24 min. *C. acerina* showed three very distinguished peaks at 30.8, 36.9, and 56.7 min, respectively. The components of *C. simplex* are most apparent in the ranges of 10−35 min. The most intense peak at 10.8 min was identified as cimifugin glucoside, which has been isolated from *C. simplex* early (*1*), by its unique mass (MW = 468) and short retention time. This compound was also observed in *C. foetida* and *C. dahurica.* It is noticeable that the molecular weights of other nine peaks of *C. dahurica* are over 800 (836−912), and their structures remained to be investigated. **Table 2** demonstrated that *C. racemosa* had the most peaks to identify and *C. simplex* had the least. Whether this discrepancy was caused by botanical reasons or by other factors is unclear. To further examine the tolerance of this method, the analytical sample solution of *C. racemosa* stored in −20 °C freezer was rechecked 1 week, 1 month, and 6 months later and their profiles were compared with their initial analyses. No significant pattern changes were observed in their profiling comparisons. This stability is as expected because no chemical was added to the final analytical samples, except methanol added as a solvent. The only chemical used in the eluate to enhance ionization is ammonium acetate, which is very mild and almost neutral. These results demonstrated that the utility of LC/MS fingerprint profiling in identifying the botanical origin of *Cimicifuga* products is a reliable and consistent method. It is expected that the LC/MS profiles of plant resources are variable depending on their growth, habitats, and harvest season. The method developed here provided a rapid and reliable way to monitor and compare the differences in their profiles therefore to identify *Cimicifuga* species by their LC/MS fingerprint library.

LC/MS Fingerprint Profile of *C. racemosa* Commercial Products. Figure 4 shows the TIS + LC/MS profiles (base peak chromatograms) of six *C. racemosa* commercial products. All of these products were claimed to originate from *C. racemosa*. Under the current optimized HPLC conditions, more than 30 distinguishable peaks were observed, and more than 10 large peaks could be used as markers for comparisons. All of these *C. racemosa*-based commercial products showed great

Table 2. Peak Identification of Some Known Compounds from *Cimicifuga* Herbs by Extract Ion Chromatograms

			plant species [a]						
compound name[b]	MW	RT	A	B	C	D	E	F	G
cimicifugenol	424						+[c]	+	+
cimifugin glucoside	468	10.8		√[c]	√				⊕[c]
dehydroxidahurinol	470	70.3							⊕
3-keto-24-epi-7,8-didehydrocimigenol	484					+			
dehydroxy-15-*O*-methyl-cimigenol	484	75.8						⊕	
24-epi-7,8-didehydrocimigenol	486	64.1, 70.1, 73.1[d]				⊕			
24-epi-acerinol	486					⊕			
7,8-didehydrocimigenol	486					⊕			
acerinol	486						+	+	+
acerionol	486						+	+	+
cimigenol (cimicifugol)	488	72.3	⊕	⊕				⊕	+
cimigol	488	72.5					⊕	+	+
dahurinol	488	66.7		⊕				⊕	+
isodahurinol	488	75.8		⊕		√	⊕	⊕	+
25-*O*-methylacerinol	500	58.5						⊕	
15-*O*-methylcimigenol	502							+	
25-*O*-methylcimigenol	502							+	+
25-*O*-methyldahurinol	502							+	
25-*O*-methylisodahurinol	502							+	
25-*O*-acetyl-7,8-didehydrocimigenol	510	79.4				⊕			
24-*O*-acetylacerionol	528	76.9					⊕	+	+
27-deoxyacetylacteol	528	64.7	√		⊕				
25-*O*-acetyl-cimigenol	530	67.0				⊕		+	
heracleifolinol	546	65.1, 71.9[e]				⊕			
foetidinol-3-*O*-xyloside	576				+				
neocimiside	576				+				
β-sitosterol-3-*O*-glucoside	576								+
15α-hydroxyfoetidinol-3-*O*-xyloside	592				+				
cimicinol	600	65.6			⊕				
cimiracemoside I	600	58.8	⊕						
25-anhydrocimicigenol-3-*O*-arabinoside	602	60.4			√		⊕		
25-anhydrocimicigenol-3-*O*-xyloside	602	69.7			⊕				
cimicidanol-3-*O*-xyloside	616				+				
cimicifugoside H-1[f]	616	**32.5**			⊕				
24-epi-7,8-didehydrocimigenol-3-*O*-xyloside	618	58.8				⊕			
7,8-didehydrocimigenol-3-*O*-xyloside	618	58.5		⊕					
cimiraceroside A	618		+						
cimicifugoside (cimigenol 3-*O*-xyloside)	620	**52.6**	⊕	⊕			⊕	⊕	⊕
16α,24α-dihydroxy-12β-acetoxy-25,26,27-trinor-16,24-cyclocycloartan-23-one 3β-*O*-arabinoside	620	52.5		⊕					
24-hydroxy-12β-acetoxy-25,26,27-trinorcycloartan-16,23-dione-3β-*O*-arabinoside	620	42.2		⊕					
actaeaepoxide 3-*O*-xyloside	620	47.2,	⊕						
cimigenol-3-*O*-arabinoside	620	58.6,	⊕	⊕		√			
cimiracemoside C	620	60.3	⊕						
shengmanol xyloside	620	60.3		⊕		√	⊕	⊕	
15α-hydroxycimicidanol-3-*O*-xyloside	632	22.3			⊕				
25-*O*-methoxylcimigenol-3-*O*-arabinoside	634	62.6	⊕						
25-*O*-methylcimigenoside	634	62.8					⊕	+	
bugbanoside F	634								+
12β-hydroxycimigenol-3-*O*-xyloside	636	21.6, 22.5, 23.0, 23.5	⊕						
12β-hydroxycimigenol-3-*O*-arabinoside	636		⊕	⊕					
cimiracemoside A	636		⊕						
cimiracemoside B	636		⊕						
22-hydroxycimigenol xyloside	638	42.6					⊕		
15α-hydroxycimicidol-3-*O*-xyloside	650	17.7			⊕				
12β,21-dihydroxycimigenol-3-*O*-arabinoside	652	17.9	⊕						
bugbanoside E	658								+
cimicifol	658	38.7			⊕				
23-epi-26-deoxyactein	660	**40.8**	⊕		⊕				
25-*O*-acetyl-7,8-didehydrocimigenol-3-*O*-xyloside	660	42.0		⊕					
3′-*O*-acetyl-24-epi-7,8-didehydrocimigenol-3-xyloside	660	56.7				⊕	√		
cimiracemoside J	660		+						
cimiracemoside K	660		+						
cimiracemoside N	660		+						
acetylshengmaol-3-*O*-xyloside	662	**70.8**	⊕	⊕			⊕	⊕	
25-*O*-acetyl-cimigenol-3-*O*-xyloside	662	**43.4**		√	⊕		⊕	⊕	
23-*O*-acetylshengmaol-3-*O*-arabinoside	662	48.4, 69.8	⊕						
25-*O*-acetyl-cimigenol-3-*O*-arabinoside	662	57.8	√	⊕					
3′-*O*-acetyl-cimigenol 3-*O*-xyloside	662	58.0				√		⊕	
cimiracemoside E	662	38.9	⊕						
bugbanoside D	674								+
cimicifugoside	674								+
cimiracemoside P	674		+						

Table 2 (Continued)

			plant species [a]						
compound name[b]	MW	RT	A	B	C	D	E	F	G
acetylacteol-3-*O*-arabinoside	676	49.7			⊕				
actein	676	39.9,	⊕		⊕				
cimiracemoside F	676	27.9,	⊕						
cimiracemoside G	676	29.2	⊕						
7,8-didehydro-24-*O*-acetylhydroshengmanol-3-*O*-xyloside	678	26.5				⊕			
(22*R*,23*R*,24*R*)-12β-acetyloxy-16β,23:22-25-diepoxy-23,24-dihydroxy-9,19-cyclolanostane-3β-yl-α-L-arabinoside	678	29.4, 34.1, 36.3, 64.2	⊕						
25-*O*-acetyl-12β-hydroxy cimigenol-3-*O*-arabinoside	678		⊕						
cimiracemoside D	678		⊕						
cimiracemoside H	678		⊕						
24-*O*-acetylhydroshengmanol xyloside	680	25.5		⊕			+	+	
bugbanoside C	692								+
2′,4′-*O*-diacetyl-24-Epi-7,8-didehydrocimigenol-3-xyloside	702	60.5, 61.0, 62.0				⊕			
2′-*O*-acetylactein	702				+				
cimiracemoside L	704	61.7,	⊕						
cimiracemoside M	704	63.6	⊕						
cimiracemoside O	718	59.1	⊕						
3-*O*-arabinosyl-cimigenol-15-*O*-glucoside	776			+					
3-arabinosyl-24-*O*-acetylhydroshengmanol-15-glucoside	842	33.8, 34.8		⊕					
3-xylosyl-24-*O*-acetylhydroshengmanol-15-glucoside	842			⊕					

[a] A, *C. racemosa*; B, *C. dahurica*; C, *C. foetida*; D, *C. heracleifolia*; E, *C. japonica*; F, *C. acerina*; and G, *C. simplex*. [b] The compounds and their distribution were summarized from the literature in refs *1–3* and *5–11*. [c] + means the compound was reported but was not detected by XIC examination; ⊕ means the compound was both reported and detected by XIC examination; √ means the compound was not be reported but was detected by XIC examination. [d] The retention times in one large combined cell are exchangeable. [e] More than one peak detected for a single mass. Not distinguishable. [f] Compound names and retention times in bold were confirmed by standard references.

Table 3. Amount of 23-Epi-26-deoxyactein in Herbs and Commercial Samples of Black Cohosh

sample	form	botanical source	23-epi-26-deoxyactein (mg)[a]	estimated % of 23-epi-26-deoxyactein in total triterpene glycosides (by peak areas)	estimated total triterpene glycosides (mg) (claims)
U.S. black cohosh	herb	*C. racemosa*	0.766/g herb	10.6%	7.2 mg/g (N/A)
herb	root and rhizome	*C. dahurica*	0.067/g herb	1.0%	6.7 mg/g (N/A)
herb	root and rhizome	*C. foetida*	0.679/g herb	8.5%	7.99 mg/g (N/A)
herb	root and rhizome	*C. heracleifolia*	tr[b]	N/A	N/A
herb	root and rhizome	*C. japonica*	tr	N/A	N/A
herb	root and rhizome	*C.acerina*	tr	N/A	N/A
herb	root and rhizome	*C. simplex*	tr	N/A	N/A
product 1	root powder in capsule	*C. racemosa*	0.143/capsule	11.2%	1.3 mg/capsule (N/A)
product 2	root powder and extract in capsule	*C. racemosa*	0.400/capsule	13.3%	3.0 mg/capsule (1 mg)
product 3	root powder in capsule	*C. racemosa*	0.098/capsule	6.2%	1.59 mg/capsule (1.0 mg)
product 4	liquid extract	*C. racemosa*	0.279/mL	7.7%	3.62 mg/mL (2 mg)
product 5	tablet	*C. racemosa*	0.057/tablet	14.9%	0.38 mg/tablet (1 mg)
product 6	sublingual tablet	*C. racemosa*	0.058/tablet	12.8%	0.45 mg/tablet (1 mg)

[a] The amount of 23-epi-26-deoxyactein was calculated based on an average of three measurements. [b] Trace, detection level not reliable.

similarities except product 3. Among them, products 1 and 2 were similar, and products 5 and 6 were similar, but there was a slight difference between these two groups of products. Product 4 was more similar to products 5 and 6 except the more intense peaks were seen in the higher elution time range. All of these samples contained 23-epi-26-deoxyactein as the largest peak except product 3. Product 3 contained more polar triterpene glycosides than other products. Interestingly, product 3 was the only one claimed to be wild-crafted and freeze-dried. Whether the difference in this product is a result of its processing method or is due to its botanical origin is unclear.

Selected Reaction Monitoring (SRM) Quantitative Analysis of 23-Epi-26-deoxyactein. Most commercial products of *C. racemosa* claim to contain triterpene glycosides calculated from 23-epi-26-deoxyactein. In this study, a LC/MS/MS method for quantitative analysis of 23-epi-26-deoxyactein was developed and performed to examine the products' claims. A selected reaction monitoring SRM experiment was chosen due to its high selectivity. **Figure 5** shows the MS/MS spectrum of 23-epi-26-deoxyactein. The fragment of m/z 452 from m/z 678 ($M + NH_4$) appeared as the most intense peak; therefore, the ion pair of m/z 678/452 was used in SRM analysis. The quantitative results were summarized in **Table 3**. The amount of total triterpene glycosides in each sample was roughly estimated from a percentage of 23-epi-26-deoxyactein in the total peak area on their base peak chromatograms by assuming all peaks over the 15−75 min retention time range were contributed by triterpene glycosides. Currently, there is no other method reported for accurately analyzing the amount of total triterpene glycosides. However, by limiting a mass range from m/z 550 to 1000, most peaks on the BPC showed molecular weights between 600 and 712 as listed in **Table 2**. These results are still estimations but

Figure 4. Base peak ion chromatograms of six commercial products.

are considered be to very close to the true values. The data in **Table 3** show that the estimated amounts of total triterpene glycosides in *C. racemosa* and *C. dahurica* are 7.2 and 6.7 mg/g, respectively. However, *C. dahurica* contained about 11 times less 23-epi-26-deoxyactein than that in *C. racemosa*. *C. racemosa* and the six *C. racemosa* commercial products contained about 6−15% of 23-epi-26-deoxyactein in total triterpene glycosides. On the other hand, the estimated amount of total triterpene glycosides in other commercial products was either greater (in products 2, 3, and 4) or lesser (in products 5 and 6) than what the manufacturers claimed.

The quality of diet supplemental products, including popular black cohosh, remains a major concern among general public and governmental agencies. Because of the complexity of constituents in such natural products and the yet to be established relationship between its constituents and claimed biomedicinal

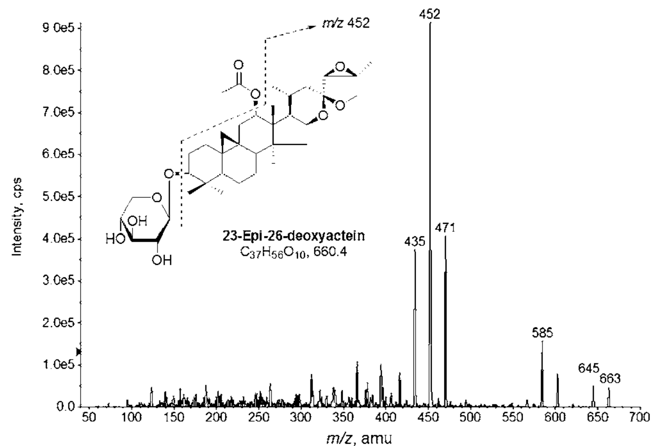

Figure 5. Product ions of m/z 678 ($M + NH_4$) of 23-epi-26-deoxyactein.

activity, the quality control of herbal products is never an easy task and adequate analysis methods are always needed.

For analyzing the constituents of *C. racemosa,* ELSD/HPLC (*9*, *10*) has been used to detect triterpene glycosides and aromatic acid esters. ELSD has an advantage to detect compounds without chromophores, but it cannot generate any structural information. APCI-LC/MS has also been used for the quantitation of phenolic and triterpenoidal compounds in *C. racemosa* (*11*). However, the fragmentation nature of APCI-mass spectra will significantly reduce the detectable sensitivity of molecular ions. For example, 23-epi-26-deoxyactein generated 12 significant fragment ions in addition to molecular ions in the mass range of m/z 350−700 (*11*). The relative intensity of the molecular ion of 23-epi-26-deoxyactein in ACPI-MS is only about 50%. Although these fragmentations can provide abundant information for structure elucidation, it is not favorable for profiling and quantitative analysis because fragmentations will significantly reduce the intensity of molecular ions and therefore diminish the detection sensitivity and quantitative accuracy of targeted compounds. TIS used in this study is one of the softest ionization methods and generates molecular ions or adduct ions as the base peak without significant fragment ions. LC/TIS-MS has been successfully applied in the analysis of ginseng triterpene glycosides (*14*). We have shown that when using TIS-MS analysis on 23-epi-26-deoxyactein, only two strong ions of $M + H$ (m/z 661.7) and $M + NH_4$ (m/z 678.5) were observed as overwhelming peaks over a mass range of m/z 5−700. This lesser fragmentation means a higher sensitivity and is favorable for profiling and quantitative analysis of targeted compounds.

The LC/TIS-MS method developed in this study is the softest ionization method, and its detection limit can be as low as 2.5 ng for 23-epi-26-deoxyactein. The structural information-rich LC/TIS-MS profiles provided a detailed fingerprint of each sample, which can be compared with others in many different ways. The methods and LC/MS profiles generated here provide a reliable and reproducible method that can be readily utilized for botanical identification of *Cimicifuga* plants, for examination and validation of its commercial products, and for "chemical" quality control in the manufacture of black cohosh products. However, a truly meaningful quality control method for a natural product such as black cohosh remains to be established by correlating some of its major constituent(s) to certain biological activities. A bioassay method, either in vitro or in vivo, which is capable of measuring estrogenic, androgenic, or other hormonal activity, may eventually be needed to establish a relationship between the chemical quality and the biomedicinal activities that are claimed by this natural product and provide a truly meaningful quality control.

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LITERATURE CITED

(1) Kusano, G. Studies on the constituents of *Cimicifuga* species. *Yakugaku Zasshi*−*J. Pharm. Soc. Jpn*. **2001**, *121*, 497−521.

(2) Compton, J. A.; Culham, A.; Jury, S. L. Reclassification of *Actaea* to include *Cimicifuga* and *Souliea* (Ranunculaceae): Phylogeny inferred from morphology, nrDNA ITS, and cpDNA *trn*L-F sequence variation. *Taxon* **1998**, *47*, 593−634.

(3) Upton, E., Ed. *Black Cohosh Rhizome*; monograph; American Herbal Pharmacopoeia: Scotts Valley, CA, 2002.

(4) Jacobson, J. S.; Troxel, A. B.; Evans, J.; Klaus, L.; Vahdat, L.; Kinne, D.; Lo, K. M. S.; Moore, A.; Rosenman, P. J.; Kaufman, E. L.; Neugut, A. I.; Grann, V. R. Randomized trial of black cohosh for the treatment of hot flashed among women with a history of cancer. *J. Clin. Oncol*. **2001**, *19*, 2739−2745.

(5) Chen, S. N.; Li, W. K.; Fabricant, D. S.; Santarsiero, B. D.; Mesecar, A.; Fitzloff, J. F.; Fong, H. H.; Farnsworth, N. R. Isolation, structure elucidation, and absolute configuration of 26-deoxyactein from *Cimicifuga racemosa* and clarification of nomenclature associated with 23-epi-26-deoxyactein. *J. Nat. Prod.* **2002**, *65*, 601−605.

(6) Watanabe, K.; Mimaki, Y.; Sakagami, H.; Sashida, Y. Cycloartane glycosides from the rhizomes of *Cimicifuga racemosa* and their cytotoxic activities. *Chem. Pharm. Bull*. **2002**, *50*, 121−125.

(7) Hamburger, M.; Wegner, C.; Benthin, B. Cycloartane glycosides from *Cimicifuga racemosa*. *Pharm. Pharmacol. Lett.* **2001**, *11*, 15−17.

(8) Hamburger, M.; Wegner, C.; Benthin, B. Cycloartane glycosides from *Cimicifuga racemosa*. *Pharm. Pharmacol. Lett.* **2001**, *11*, 98−100.

(9) Li, W. K.; Chen, S. N.; Fabricant, D.; Fabricant, D.; Angerhofer, C. K.; Fong, H.; Farnsworth, N. R.; Fitzloff, J. F. High-performance liquid chromatographic analysis of black cohosh (*Cimicifuga racemosa*) constituents with in-line evaporative light scattering and photodiode array detection. *Anal. Chim. Acta* **2002**, *471*, 61−75.

(10) Ganzera, M.; Bedir, E.; Khan, I. A. Separation of *Cimicifuga racemosa* triterpene glycosides by reversed phase high performance liquid chromatography and evaporative light scattering detection. *Chromatographia* **2000**, *52*, 301−304.

(11) He, K.; Zheng, B.; Kim, C. H.; Rogers, L.; Zheng, Q. Direct analysis and identification of triterpene glycosides by LC/MS in black cohosh, *Cimicifuga racemosa*, and in several commercially available black cohosh products. *Planta Med.* **2000**, *66*, 635−640.

(12) Li, W. K.; Sun, Y. K.; Liang, W. Z.; Fitzloff, J. F.; van Breemen, R. B. Identification of caffeic acid derivatives in *Actea racemosa* (*Cimicifuga racemosa*, black cohosh) by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 978−982.

(13) Onorato, J.; Henion, J. D. Evaluation of triterpene glycosides estrogenic activity using LC/MS and immunoaffinity extraction. *Anal. Chem.* **2001**, *73*, 4704−4710.

(14) Wang, X.; Sakuma, T.; Asafu-Adjaye, E.; Shiu, G. K. Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. *Anal. Chem.* **1999**, *71*, 1579−1584.

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