

Structural Determination of Ginsenosides Using MSⁿ Analysis

Linda L. Lopez

Introduction

Ginseng root, a traditional Chinese herbal remedy, contains more than a dozen biologically active saponins called ginsenosides. This class of natural products is believed to play an important role in the treatment and prevention of a number of diseases including atherosclerosis, arthritis, asthma, diabetes, stroke, multiple sclerosis, and endotoxin liver injury.^{1–3} Ginsenosides are among a growing class of herbal and vitamin products known as nutraceuticals, that is, food products that have pharmacological benefits to human health because of their therapeutic properties. With an estimated 15 million patients at risk of potentially adverse drug-herb interactions,⁴ there is renewed interest in the isolation and characterization of these compounds.

Ginsenosides are structurally described as glycosides consisting of an aglycone moiety, which is typically a triterpenoid or steroid, and one or more covalently linked sugar monomers. Since most ginsenosides contain multiple oligosaccharide chains at different positions in the molecule, structural elucidation of these compounds can be quite complicated. Tandem mass spectrometric methods have been developed for the characterization of ginsenosides contained in ginseng extracts.⁵ However, MS/MS experiments carried out on a triple quadrupole mass spectrometer using a collision cell typically generate complex product ion spectra that are often difficult to interpret. This is because first-stage product ions tend to undergo further collisions with the background gas to yield second and third generation fragments that cannot be easily distinguished from first-stage MS/MS product ions.

MSⁿ analysis in an ion trap mass spectrometer permits multiple isolation and fragmentation stages, ensuring that product ions in each stage are specifically related to the precursor ion from that particular stage. This type of stepwise fragmentation can be quite advantageous because it allows product ion origins to be unambiguously assigned, making MS/MS spectra simpler to interpret and permitting individual fragmentation pathways to be followed.

This note demonstrates the power of MSⁿ analysis for the structural determination of ginsenosides from a ginseng root extract.

Experimental

All experiments were done using an Agilent 1100 Series LC/MSD Trap system composed of a binary pump, vacuum degasser, autosampler, and thermostatted column compartment with column-switching valve. The system was operated with the electrospray ionization (ESI) source in the positive ion mode.

Reagent grade chemicals and HPLC grade solvents were used in preparing mobile phases and standards.

Results and Discussion

Figures 1a–c show the full scan MS, MS/MS and MS³ spectra from direct infusion of the Rb1 ginsenoside standard, along with proposed origins of the observed product ions. The mass spectrum of Rb1 (Figure 1a) shows predominantly the intact [M+Na]⁺ pseudomolecular ion at *m/z* 1131.7, with little or no decomposition of the labile ginsenoside adduct under typical ESI interface conditions using a drying gas temperature of 350°C. This is in contrast to previous studies in which a room temperature API interface was required to observe an intact molecular ion,⁵ and emphasizes the gentle nature of the orthogonal spray ion source on the LC/MSD Trap.

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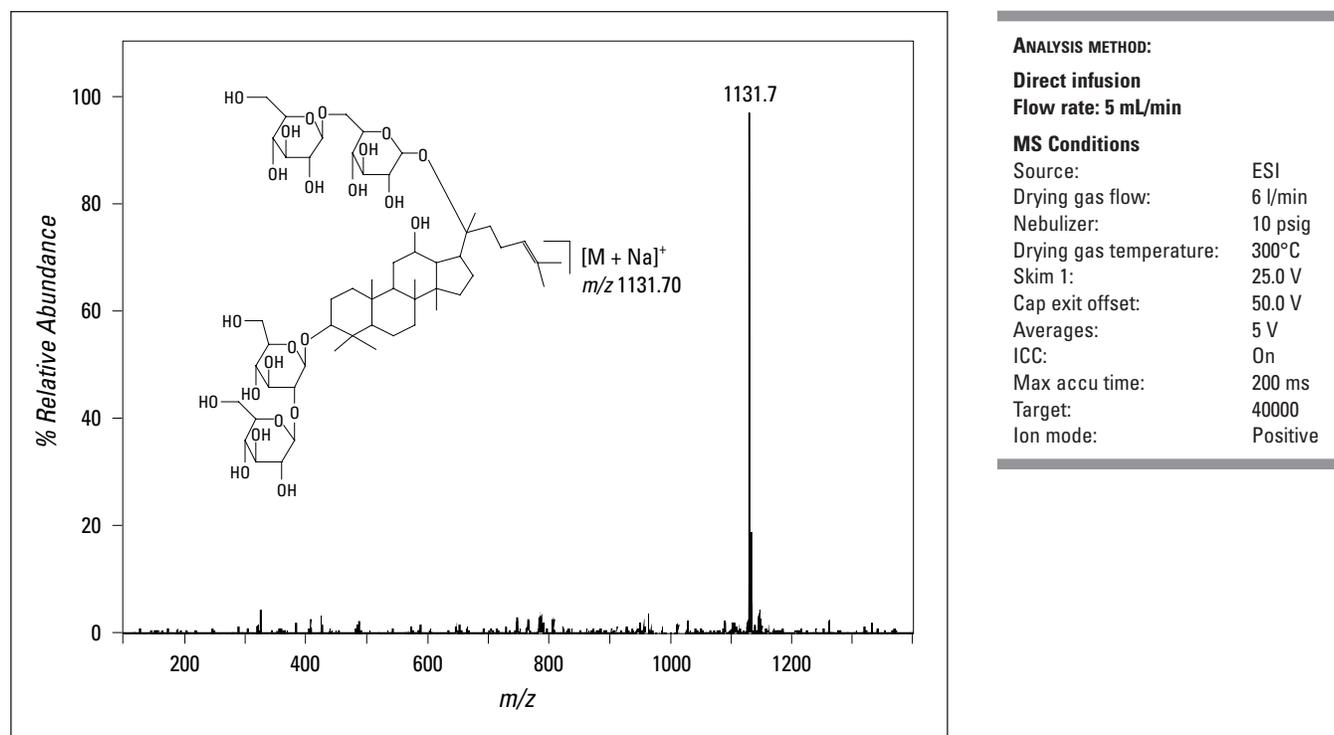


Figure 1a. Full scan mass spectrum of ginsenoside Rb1 standard.

MS/MS of the m/z 1131.7 sodium adduct shown in Figure 1b yields a product ion at m/z 789.6 corresponding to cleavage of a single glycosidic bond. For the case of the ginsenoside Rb1, which contains two isomeric oligosaccharide chains, cleavage at either glycosidic linkage would result in isobaric fragment ions at m/z 789.6. Subsequent isolation and fragmentation of m/z 789.6 (Figure 1c) yields two products: (1) a more abundant ion corresponding to loss of the oligosaccharide chain ($-\text{Glc}^2-\text{Glc}$) at m/z 365.1 and (2) loss of a deoxyhexose sugar at m/z 627.5. The stepwise fragmentation observed in the ion trap provides specific information on molecular structure; however, in this case it is not possible to establish the initial site of glycosidic bond cleavage in the molecule.

For use as a reference in the interpretation of ginsenoside Rb1 fragmentation spectra, several analogs of ginsenoside Rb1 were analyzed by ion trap LC/MSⁿ. In this manner an MS/MS comparative method was devised based on the premise that ginsenoside Rb1 would be expected to retain substructures of the related ginsenosides. For example, Figure 2 shows the

total ion chromatogram generated from online LC/MS³ analysis, as well as extracted ion chromatograms of the pseudomolecular ions of each chromatographic peak generated from a water extract of American ginseng root. Figures 3a and 3b show the online product ion spectra of the ginsenoside Rb2 which contains two different oligosaccharide chains. Isolation and fragmentation of the pseudomolecular ion at m/z 1101.6 yields a single ion at m/z 789.7 resulting from cleavage of the $-\text{Glc}^6-\text{Ara}(\text{p})$ disaccharide linkage. Comparison of the product ion spectra of ginsenosides Rb1 with Rb2 shows isobaric product ions (m/z 789.7), thus supporting the proposed fragmentation pattern for ginsenoside Rb1 shown in Figure 1. Furthermore, m/z 789.7 was a major fragment ion observed in the product ion spectra of all the ginsenosides analyzed, providing a substructural template that supports the fragmentation patterns proposed for Rb1 and Rb2.

Subsequent isolation of the m/z 789.7 precursor generates a full scan MS³ spectrum, which is shown in Figure 3b along with proposed origins of the observed product ions.

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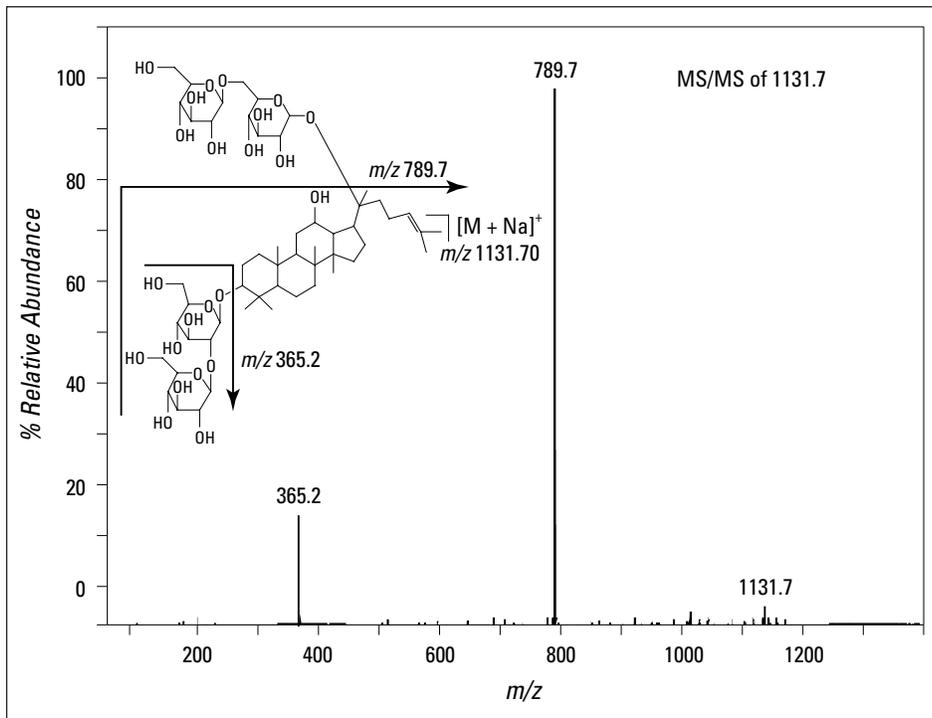


Figure 1b. Product ion spectrum generated from infusion of ginsenoside Rb1 standard by MS/MS analysis of m/z 1131.7 ($[M+Na]^+$).

ANALYSIS METHOD:

Direct infusion
Flow rate: 5 mL/min

MS Conditions

Source:	ESI
Drying gas flow:	6 l/min
Nebulizer:	10 psig
Drying gas temperature:	300°C
Skim 1:	25.0 V
Cap exit offset:	50.0 V
Averages:	5 V
ICC:	On
Max accu time:	200 ms
Target:	40000
Ion mode:	Positive

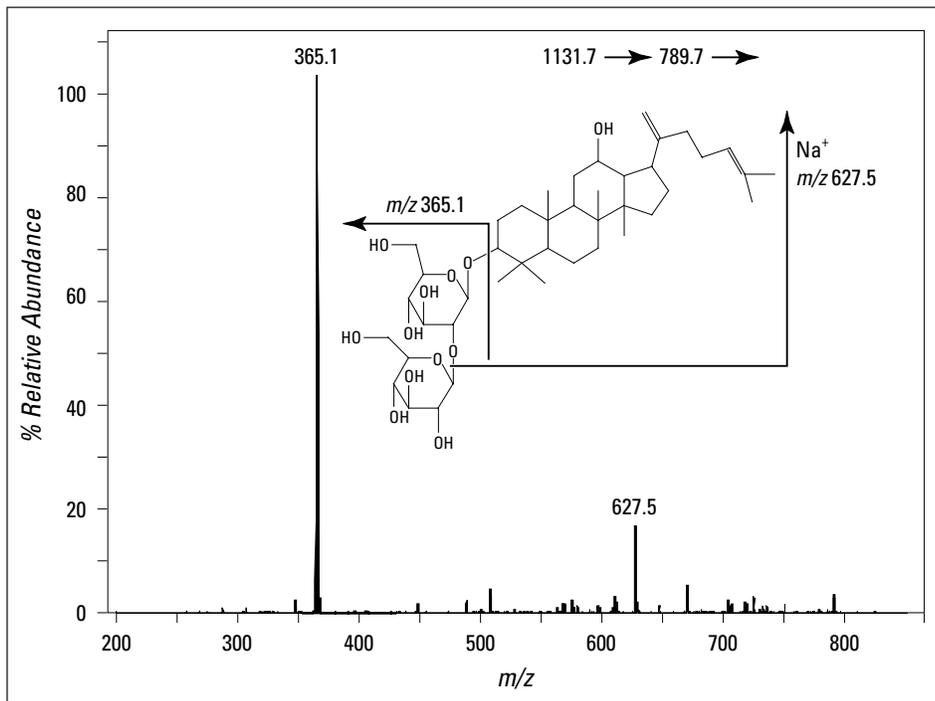


Figure 1c. Product ion spectrum generated from infusion of ginsenoside Rb1 standard by MS³ analysis of m/z 789.7 (from m/z 1131.7).

ANALYSIS METHOD:

Direct infusion
Flow rate: 5 mL/min

MS Conditions

Source:	ESI
Drying gas flow:	6 l/min
Nebulizer:	10 psig
Drying gas temperature:	300°C
Skim 1:	25.0 V
Cap exit offset:	50.0 V
Averages:	5 V
ICC:	On
Max accu time:	200 ms
Target:	40000
Ion mode:	Positive

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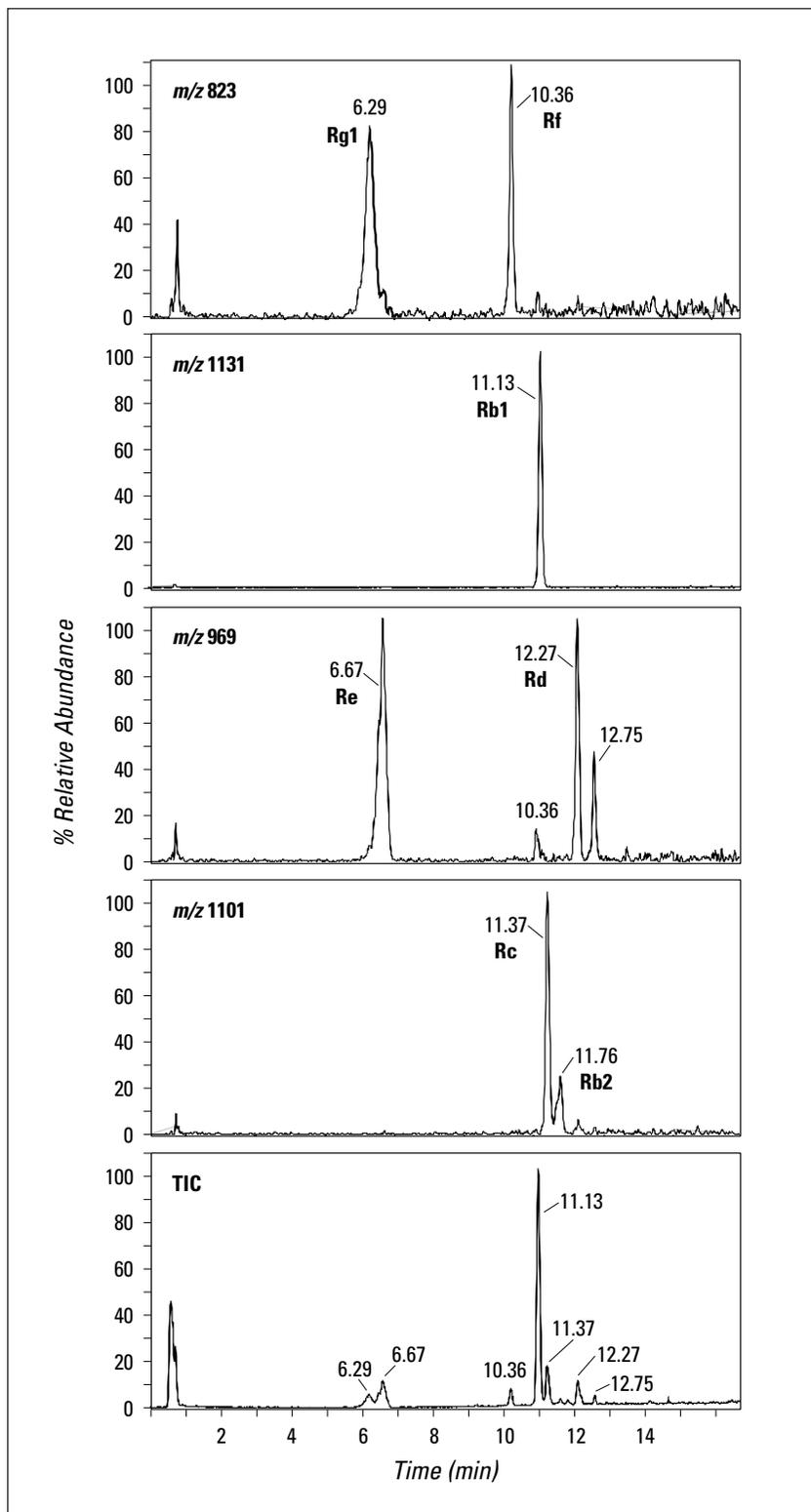


Figure 2. Total ion chromatogram (TIC) and extracted ginsenoside pseudomolecular ion chromatograms generated from LC/MSⁿ analysis of ginseng root extract.

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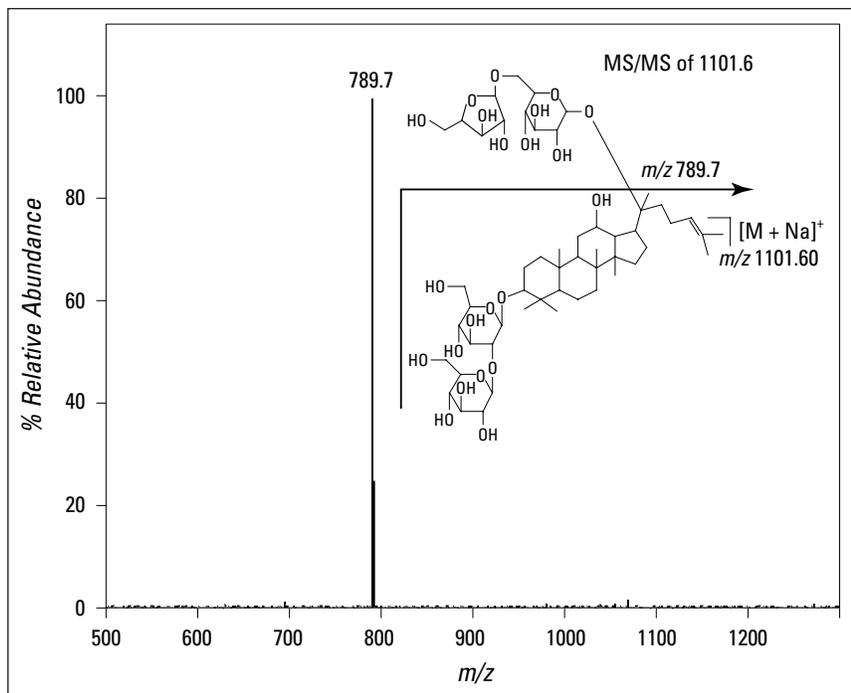


Figure 3a. Product ion spectrum generated from ginseng root extract by LC/MSⁿ analysis of m/z 1101.6 ($[M+Na]^+$).

ANALYSIS METHOD:

Chromatographic Conditions

Column: 2 × 50 mm Zorbax® SB-C18,
3.5 μm (p/n 863954-302)
Mobile phase: A = 0.01% acetic acid
in water;
B = acetonitrile
Gradient: start with 20% B
at 1 min 20% B
at 11 min 95% B

Flow rate: 0.3 ml/min
Injection volume: 10 μl

MS Conditions

Source: ESI
Drying gas flow: 7 l/min
Nebulizer: 30 psig
Drying gas
temperature: 300°C
Skim 1: 25.0 V
Cap exit offset: 50.0 V
Averages: 5 V
ICC: On
Max accu time: 200 ms
Target: 40000
Ion mode: Positive

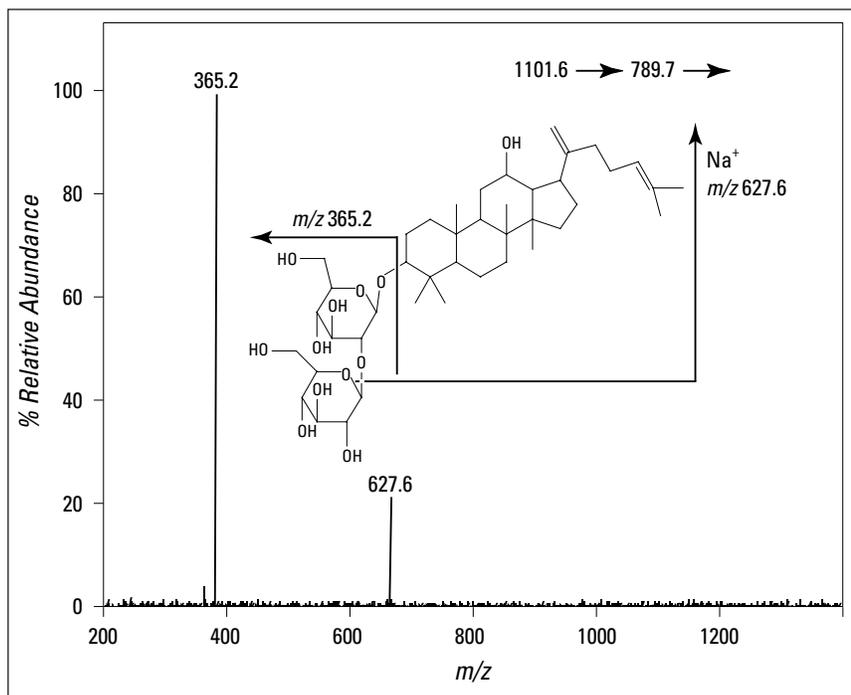


Figure 3b. Product ion spectrum generated from ginseng root extract by LC/MSⁿ analysis of m/z 789.7 (from m/z 1101.6).

ANALYSIS METHOD:

Chromatographic Conditions

Column: 2 × 50 mm Zorbax® SB-C18,
3.5 μm (p/n 863954-302)
Mobile phase: A = 0.01% acetic acid
in water;
B = acetonitrile
Gradient: start with 20% B
at 1 min 20% B
at 11 min 95% B

Flow rate: 0.3 ml/min
Injection volume: 10 μl

MS Conditions

Source: ESI
Drying gas flow: 7 l/min
Nebulizer: 30 psig
Drying gas
temperature: 300°C
Skim 1: 25.0 V
Cap exit offset: 50.0 V
Averages: 5 V
ICC: On
Max accu time: 200 ms
Target: 40000
Ion mode: Positive



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Conclusions

MSⁿ analysis using an ion trap mass spectrometer specifically selects the desired precursor ion and dissociates it to produce a specific fragmentation pattern in individual stages. As a result, it is a powerful analytical tool for deducing molecular structure. Electrospray ionization provides a soft ionization technique for generating predominantly intact molecular or pseudomolecular ions with little or no structurally relevant fragment ions in the mass spectra. MS/MS fragmentation in an ion trap mass spectrometer is useful because the product ions generated are derived only from the original molecular ion and are not the result of any additional fragmentation, as can be the case with collision induced dissociation (CID) in a collision cell. Additional MS stages tend to show stepwise fragmentations in which all or most of the ion current is localized in a single product ion, greatly facilitating interpretation of the spectra.

References

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