# Application Note 01119

## Structural Elucidation of Ginsenosides Using the Varian 500-MS Ion Trap Mass Spectrometer

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### Introduction

Ginsenosides are active components of Panax ginseng[1]. Among the 30 ginsenosides identified so far, ginsenosides Rb1, Rb2, Rc, Re and Rd are the most abundant and are associated with the pharmacological activity of the plant material[2]. Since ancient times, traditional Chinese medicine (TCM) has been used in the treatment of atherosclerosis, arthritis, asthma, diabetes, stroke, multiple sclerosis and endotoxin liver injury[3]. Ginseng root (Panax ginseng) is the major ingredient in TCM. Active components of ginseng root are ginsenosides that fall under the category of nutraceuticals (food components/products that have therapeutic and pharmacological properties). With an estimated 15 million patients at risk of potentially adverse drug-herb interactions, there is a renewed interest in the analysis of these compounds.

The main problem in the analysis of ginsenosides is their structural similarity. They are described as glycosides consisting of an aglycone moiety that is typically a triterpenoid or steroid and one or more covalently linked sugar monomers. Most ginsenosides contain multiple oligosaccharide chains at different positions. Analysis of these compounds can therefore be quite complicated. ESI-TOF, triple quadrupole MS/MS and Ion trap $MS^n$ methods have been developed so far for the structural elucidation and analysis of these compounds in complex matrices. Among these methods, $MS^n$ 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. It is possible to confirm the authenticity of the pharmaceutical ginseng products and differentiate between their active ingredients using ion trap $MS^n$ fragmentation patterns.

This application note demonstrates the use of an LC/$MS^n$ method for the structural elucidation of ginsenosides Rb1, Rb2, Rc, Re and Rd on the Varian 500-MS Ion Trap LC/MS. This method was validated in Korean ginseng extract. The information obtained from the ion trap $MS^n$ and MRM could be used for determining the structure of ingredients and for quality control purposes in complex matrices.

### Instrumentation

- Varian 500-MS LC Ion Trap with an ESI source
- Varian 212-LC Binary Gradient LC/MS Pumps
- HTS Pal AutoSampler

### Materials and Reagents

Ginsenosides Re, Rc, Rd, Rb1 and Rb2 were obtained from Chromadex Inc, Santa Ana, CA. HPLC grade water and acetonitrile were obtained from Fisher Scientific.

### Sample Preparation

Powdered Korean ginseng (0.3 g) was ultra sonicated in 6 mL methanol for 30 minutes. The extract was filtered initially using Whatmann #1 filter paper. This was further filtered using a 0.2 µm syringe filter.

### LC Conditions

Column: Polaris™ C18-A 3 µm, 150 x 2 mm ID (Varian Part Number A2001150X020)
Buffer A: Water
Buffer B: Acetonitrile
Injection Volume: 50 µL

LC Program:

| Time (min:sec) | %A | %B | Flow (µL/min) |
|---|---|---|---|
| 0:00 | 75.0 | 25.0 | 200.0 |
| 40:00 | 60.0 | 40.0 | 200.0 |
| 40:01 | 75.0 | 25.0 | 200.0 |
| 50:00 | 75.0 | 25.0 | 200.0 |

### API Conditions

Ionization Mode: ESI Positive
API Drying Gas: 15 psi at 350 °C
API Nebulizing Gas: 25 psi
Needle: 4000 V
Shield: 600 V
Detector: 1400 V

### TurboDDS™ Scan Conditions

Scan Type: TurboDDS
Survey Scan Start Mass: 900 *m/z*
Survey Scan End Mass: 1200 *m/z*
RF Loading: 100%
$MS^n$ Depth: n = 3
Auto Mass Range: Yes
Isolation Window: 3.0 *m/z*

An exclusive include list containing molecular masses of all five ginsenosides and their respective $MS^2$ and $MS^3$ fragments was created. TurboDDS™ was ordered to trigger the masses given in the include list based on abundance. The exclusive include abundance list for all five ginsenosides is given in Table 1.

Table 1. Exclusive include abundance list for all five ginsenosides.

| Ginsenosides | $MS^1$ | $MS^2$ | $MS^3$ |
|---|---|---|---|
| Re | 967.0– 972.0 | 787.0 – 791.0 | 347.0 – 350.0 |
| Rb1 | 1130.0 – 1133.0 | 787.0 – 791.0<br>363.0 – 366.0 | 363.0 – 366.0 |
| Rc/Rb2 | 1100.0 – 1102.0 | 787.0 – 790.0<br>333.0 – 336.0 | 363.0 – 366.0 |
| Rd | 967.0 – 972.0 | 787.0 – 791.0 | 363.0 – 366.0 |

## Results & Discussion

Initially, all five ginsenosides (Rc, Re, Rb1, Rb2 and Rd) were infused at 300 pg/µL concentrations on the Varian 500-MS. MS/MS and $MS^3$ data was acquired automatically using the TurboDDS™ software in a data dependent fashion to investigate in detail the structural elucidation of these ginsenosides. Even though ginsenosides ionize well in the negative ion mode under normal electrospray conditions, structural elucidation cannot be done at the $MS^2$ level since all five ginsenosides are similar. In the positive ESI mode protonated sodiated adduct ions $[M+Na]^+$ are formed when high drying gas temperatures are applied (350 °C). $MS^2$ and $MS^3$ analysis of these ions gave much clearer fragmentation, thus facilitating structural elucidation.

The fragmentation pattern of ginsenoside Re on the 500-MS using the TurboDDS software is given in Figure 1. The main ion observed for ginsenoside Re in the positive mode is [M+H] + Na = 969.2. $MS^2$ fragmentation yielded a single product ion at *m/z* = 789.1 that was produced by the loss of a glucose molecule from the sodiated molecular ion 969.2. At the $MS^3$ level, the $MS^2$ product ion 789.1 is cleaved into two parts. The detected part at $MS^3$ level is *m/z* = 348.8, which comes from the cleaved disaccharide moiety.

Ginsenoside Rd has the same sodiated protonated molecular ion (*m/z* = 969.1) and $MS^2$ product ion (*m/z* = 789.2) as ginsenoside Re. Interestingly, ginsenoside Rd could be differentiated from ginsenoside Re by the fragmentation at $MS^3$ stage on 500-MS. Fragmentation of ginsenoside Rd yielded a disaccharide ion at *m/z* 364.9 at the $MS^3$ stage (Figures 1 and 2).

The main precursor ion observed for ginsenoside Rb1 is at *m/z* 1131.3, and is subsequently fragmented in the MS/MS experiment into two product ions at *m/z* 789.3 and *m/z* 364.9 (Figure 3). At the $MS^3$ level, *m/z* 789.3 is further fragmented to yield a disaccharide ion at *m/z* 364.8.

Isomeric ginsenosides Rc and Rb2 have the same fragmentation patterns. Two fragments were obtained at the $MS^2$ level, one at *m/z* = 789.2 and the other at 334.9 for the arabinose disaccharide. The other disaccharide *m/z* 364.8 is cleaved off from the ion 789.2 at the $MS^3$ level (Figure 4).

All five ginsenosides analyzed have the same $MS^2$ level main product ion corresponding to *m/z* = 789.1. This provides a structural template that supports the fragmentation patterns proposed for these compounds.

As the saccharide molecules connected to the triterpene structures of ginsenosides are cleaved off in a characteristic pattern with the ion trap MS/MS and $MS^n$, it is possible to differentiate ginsenosides in ginseng extract. If the specific fragmentation pattern of ginsenosides is known, it helps to detect the presence of ginsenosides and specifically ginseng root in complex natural product extracts.

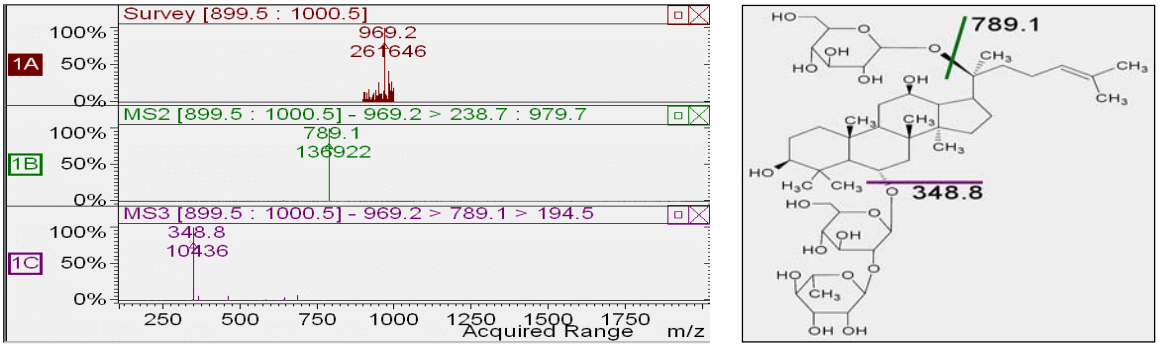


Figure 1. Ginsenoside Re fragmentation on 500-MS (left). Structure of ginsenoside Re (right).

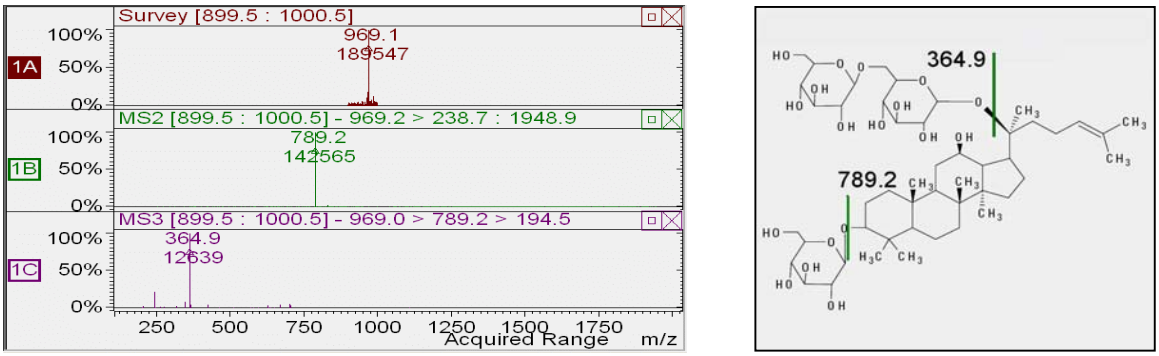


Figure 2. Ginsenoside Rd fragmentation pattern on 500-MS (left). Structure of ginsenoside Rd (right).

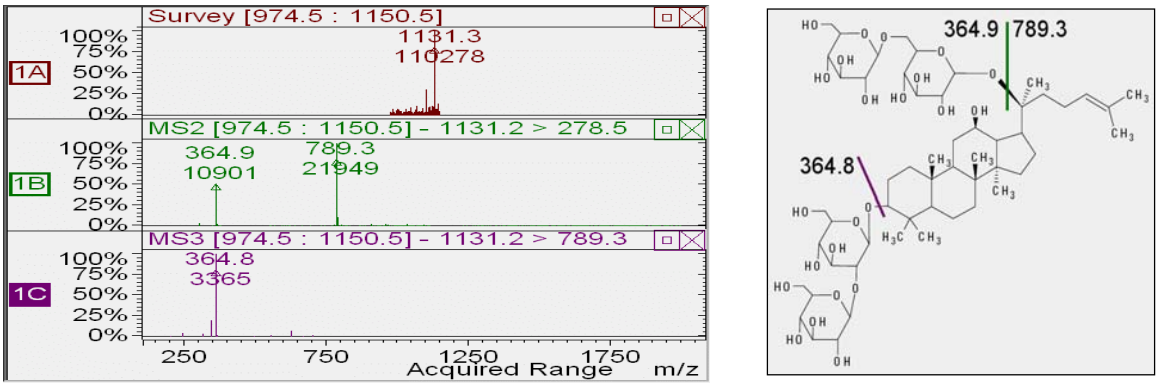


Figure 3. Ginsenoside Rb1 fragmentation pattern on 500-MS (left). Structure of ginsenoside Rb1 (right).

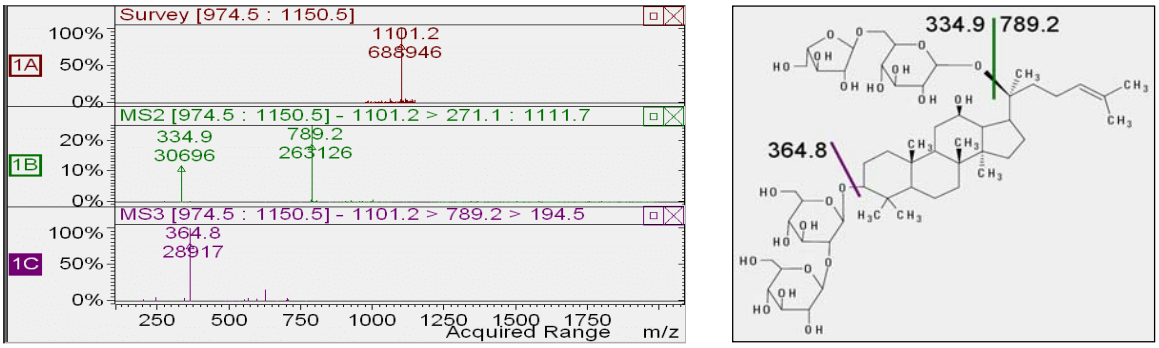


Figure 4. Ginsenoside Rc/Rb2 fragmentation pattern on 500-MS (left). Structure of ginsenoside Rc/Rb2 (right).

In the next step, ginsenosides Re, Rc, Rb1, Rb2 and Rd were separated chromatographically using a Varian Polaris™ C18-A 150 x 2.0 mm ID column. Product ions obtained by the fragmentation of sodium adducts in the positive ion mode were used for monitoring MRM transitions. Excellent separation with good resolution was obtained for all five ginsenosides (Figure 5).

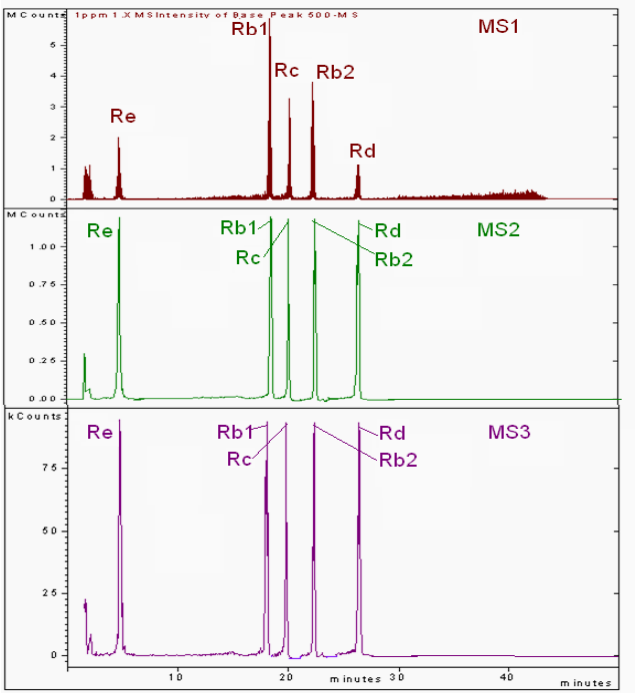


Figure 5. Extracted ion MRM chromatograms of five ginsenosides.

Chromatographic retention times of ginsenosides and their respective MRM transitions are given in Table 2. As seen from the table, chromatographic retention times along with $MS^2$ and $MS^3$ product ions specific for each ginsenoside could be used for generating qualitative fingerprints of these compounds in complex extracts.

Table 2. MRM transitions and LC retention times of ginsenosides studied.

| Name | Retention Time (min) | Parent Ion | $MS^2$ Product Ions | $MS^3$ Product Ions |
|---|---|---|---|---|
| Ginsenoside Re | 4.751 | 969.2 | 789.1 | 348.8 |
| Ginsenoside Rb1 | 18.485 | 1131.3 | 789.3, 364.9 | 364.8 |
| Ginsenoside Rc | 20.082 | 1101.2 | 789.2, 334.9 | 364.8 |
| Ginsenoside Rb2 | 22.086 | 1101.2 | 789.2, 334.9 | 364.8 |
| Ginsenoside Rd | 26.317 | 969.1 | 789.2 | 364.9 |

In the next step, Korean ginseng extract was tested for the presence of these ginsenosides. 0.3 g of Korean ginseng powder was extracted into 6 mL methanol. This extract was diluted 500 times with 25% acetonitrile before injection on the 500-MS. As shown in Figure 6, all five ginsenosides were detected in the extract. $MS^2$ and $MS^3$ spectra extracted from the peaks further confirmed their presence thus generating their fingerprint.

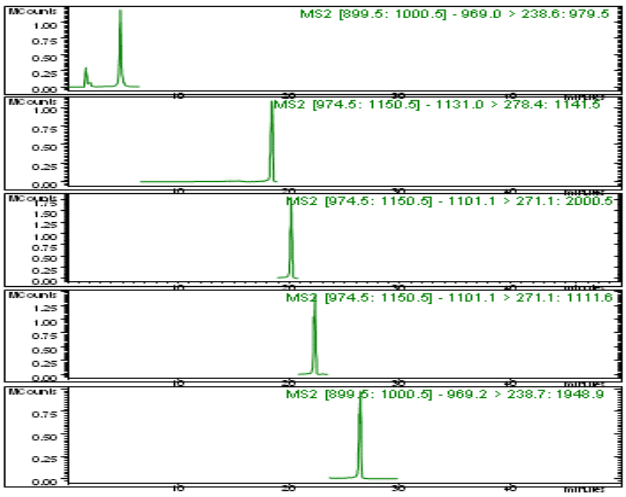


Figure 6. Five ginsenosides observed in the Korean ginseng extract.

## Conclusion

This application note successfully demonstrates the use of the Varian 500-MS Ion Trap mass spectrometer with its powerful TurboDDS™ software as an ideal tool for the structural elucidation of natural products in complex natural extracts. Complex structures of five ginsenosides, which are the main components of natural extracts, could be elucidated by the interpretation of the MS/MS and $MS^n$ ion trap data obtained. Detailed knowledge of fragmentation data on an ion trap could be used for the quality control of complex natural extracts or pharmaceutical products by ion trap $MS^n$ when applied to structurally similar active ingredients in natural products.

## References

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2. Shibata, S.; Tanaka, O.; Shoji, J.; Saito, H. *Economic and Medicinal Plant Research.* 1985, Vol 1.
3. Kim, Y.C.; Kim, S.R.; Markelonis, G.J.; Oh, T.H. *J Neurosci. Res.* 1998, 53, 1998, 426-32.
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