Analysis of a complex natural product extract from ginseng – Part II: Structure elucidation of ginsenosides by high resolution ion trap LC/MS

Application Note

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Abstract

Since prehistoric time extracts from herbs have been used for medical treatment of disease. Their activity and effects on humans were found by trial and error over generations. A good example of achieved efficiency is traditional Chinese medicine (TCM). In Western medicine drugs derived from natural origins are gaining importance due to their potential. However, Western pharmaceutical quality standards require a deep knowledge about the ingredients in medication based on natural products. This Application Note will demonstrate the use of the Agilent 1200 Series Rapid Resolution LC system with Rapid Resolution High Throughput (RRHT) columns for the separation of the ingredients found in a complex ginseng root extract. The information obtained with an ion trap MS^n and MRM is used to determine the structure of the ingredients and for quality control purposes.

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Introduction

Crude extracts of herbal and animal origin have been used for medical treatment of disease in all ancient cultures around the world since prehistoric time. Their activity for treatment of different diseases and other effects on humans were found by trial and error over hundreds of years and the knowledge about this medicine was passed on from generation to generation. A good example of the efficiency achieved during this process of optimization is the herbal based traditional Chinese medicine (TCM). A famous Asian herb, which has been used in herbal medicine for more than 5000 years is the ginseng root (*Panax ginseng*). The main active compounds of the ginsenosides are triterpene saponins of which more than 80 have been isolated and characterized during the past years. A lot of work was done during the last 30 years to develop analytical methods for the analysis of ginseng extracts and medical formulations. The method of choice for the analysis of complex natural product extracts such as those derived from the ginseng root is high performance liquid chromatography (HPLC)[1]. LC/MS equipment, e.g LC/ESI oaTOF for accurate mass measurement and ion trap LC/MS or triple quadrupole LC/MS instruments for structure elucidation by MS/MS and MS^n are currently used to determine the complex and similar structures of ginsenosides[2]. In particular, it is possible to confirm the authenticity of the pharmaceutical ginseng products and differentiate between their active ingredients using the ion trap MS^n fragmentation patterns[3].

This Application Note will demonstrate the Agilent 1200 Series Rapid Resolutioin LC system with 1.8-µm columns for the separation of the ingredients found in a complex ginseng root extract. The information obtained with an ion trap MS^n and MRM is used to determine the structure of the ingredients and for quality control purposes. The use of a high resolution LC system together with an ESI oaTOF for accurate mass measurement is described in Part I in this series of Application Notes[4].

Experimental

Equipment

- Agilent 1200 Series binary pump SL with a degasser. This pump has the capability to perform high resolution HPLC analysis on a 1.8 -µm particle size RRHT column and achieve the best performance.
- Agilent 1200 Series high performance autosampler SL with a thermostat. This autosampler is especially designed to work with the Agilent 1200 Series binary pump SL to ensure lowest delay volumes.
- Agilent 1200 Series thermostatted column compartment (TCC). The TCC is ready for use with the Agilent 1200 Series binary pump SL and optional separate heat exchangers, as well as post column cooling, under optimized delay volume conditions, together with alternating column regeneration with an optional 2-position/10-port valve.
- Agilent 1200 Series diode array detector SL (DAD). This DAD is capable of acquiring data with a sampling rate of up to 80 Hz. In case of network problems, this device has a built-in data storage capability.
- Agilent 6330 Ion Trap LC/MS.The ion trap is operated with a standard ESI source and able to scan with 26,000 m/z /sec. The MS^n spectra are acquired data dependent and fully automated.
- The software used for instrument control was ChemStation B01.03, ion trap software 5.3, and for data analysis the ion trap data analysis software 3.3.
- Column: ZORBAX SB C18, 2.1 x 150 mm, 1.8 µm

Sample

Powdered freeze-dried Asian ginseng root (1g) (*Panax ginseng*) was treated ultrasonically for 30 minutes in 10 mL methanol, filtered and directly used for analysis.

The set-up of the Agilent 6330 Ion Trap LC/MS system is shown in figure 1. The Agilent 1200 Series binary pump SL is connected to the Agilent 1200 Series high performance autosampler SL (ALS SL) with a 0.17-mm i.d. stainless steel capillary. To reduce delay volume, the seat capillary in the ALS SL has an i.d of 0.12 mm. The same kind of capillary connects to the low delay volume (1.6 µL) heat exchanger in the Agilent 1200 Series thermostatted column compartment, which is connected to the column. A 2-µL cell is built into the Agilent 1200 Series diode-array detector SL for UV detection. The outgoing capillary is directly connected to the sprayer of the electrospray source of the 6330 Ion Trap LC/MS, which is capable of acquiring data with a scan speed of 26,000 m/z/sec. This instrument set-up is optimized to achieve the highest possible resolution, which is demonstrated

by the UV analysis of a complex natural product extract obtained from Asian ginseng root (*Panax ginseng*). To illustrate the performance, a comparative analysis on an Agilent 1100 Series LC system (5-µm particle size column) and on an Agilent 1200 Series Rapid Resolution LC system (1.8-mm particle size column) is presented[4]. The resulting UV chromatograms acquired at 220 nm clearly demonstrate the better resolution of the peaks on the Agilent 1200 Series Rapid Resolution LC system. The peak width (FWHM) of the majority of the peaks in the UV chromatogram is below 0.1 min with baseline separation. The full performance of the Agilent 1200 Series Rapid Resolution LC system in the high resolution configuration is documented in a separate Application Note[5]. It is also possible to use this system, with minor changes, in a high throughput environment, which is described in another Application Note[6].

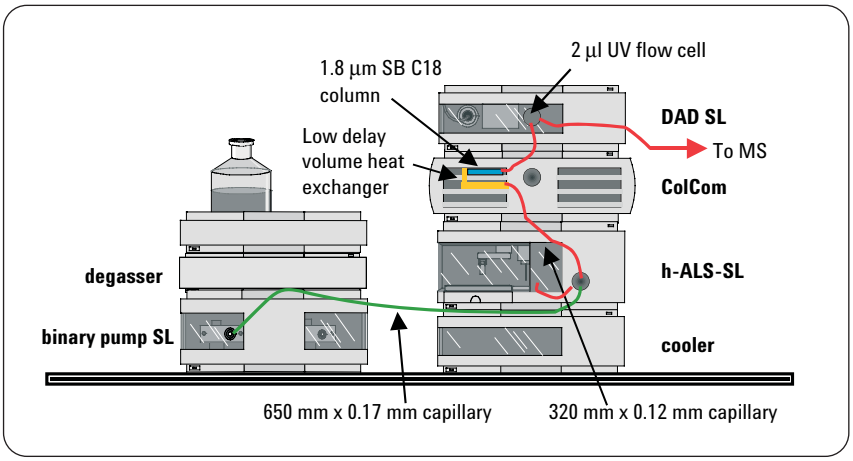

Figure 1
Agilent 1200 Series Rapid Resolution LC system for MS in low delay volume configuration.

Methods

- The Agilent 1200 Series binary pump SL was operated under the following conditions:
  Solvent A: water + 0.1 % TFA
  Solvent B: AcN + 0.1 % TFA
  Flow: 0.5 mL/min
  Gradient: 0 min 5 % B,
  1 min 5 % B,
  60 min 85 % B,
  61 min 95 % B,
  70 min 95 % B
  Stop time: 70 min
  Post time: 15 min
- The Agilent 1200 Series high performance autosampler SL was used for injections of 10 µL sample and the samples were cooled to 10 °C. The sample loop was switched to bypass after 1 minute to reduce delay volume.
- The Agilent 1200 Series thermostatted column compartment SL was adjusted to 50 °C equipped with the low delay volume heat exchanger.
- The Agilent 1200 Series diode-array detector SL was operated at 80 Hz for data acquisition at a wavelength of 220 nm/4, ref. 360/100 with the 2-µL flow cell, 30-mm path length.
- The 6330 Ion Trap LC/MS was operated under the following conditions:
  Source: ESI in positive mode.
  Dry gas: 5.0 L/min
  Dry temp.: 300 °C
  Nebulizer: 15 psi
  Target: 125,000
  Max. accum. time: 100 ms
  Scan: 200-1300
  Averages: 2
  MS^n: Automated MS/MS and MS^3

Results and discussion

After separation of the individual compounds, which are components of the crude extract, from the ginseng root by means of high resolution HPLC on a 1.8-µm particle size column, they are subjected to fragmentation for MS^n. The major ingredients elute between 20 and 33 minutes and are recorded as ginsenosides Re, Rf, F_{11}, Rb_1, Rb_2, Rc, Rd in the base peak chromatogram (figure 2). The high resolution of the column used resolves a large amount of minor compounds from the ginseng extract, which may also be analyzed because the high scan rate of 26,000 m/z/sec allow sufficient ion trap MS/MS and MS^n data to be acquired. The ion trap MS/MS and MS^3 data of the ginsenosides Re, Rb_1 and Rc were investigated in more detail for structure elucidation while the species-dependent ginsenosides Rf and F_{11} is discussed in another part of this study[7].

The simple MS scan delivers the mass of the molecular ion in its protonated and sodiated form (figure 3). The ratio of protonated and sodiated ions depends on the electrospray source temperature because the sodiated complexes are more stable at higher temperatures than the protonated ions, which decompose due to a loss of water and other fragmentations. The MS scan delivers the ions at m/z 928.9 $[M+H-H_2O]^+$, 946.9 $[M+H]^+$, 969.1 $[M+Na]^+$ for ginsenoside Re; at m/z 1090.1 $[M+H-H_2O]^+$, 1108.5 $[M+H]^+$, 1131.1 $[M+Na]^+$ for ginsenoide Rb_1 and at m/z 1059.71 $[M+H-H_2O]^+$, 1077.75 $[M+H]^+$, 1101.1 $[M+Na]^+$ for ginsenoside Rc.

The conditions for the MS/MS and MS^3 fragmentation in the 6330 Ion Trap LC/MS were essentially chosen to produce sodiated ions. The fragmentation of these ions gave much clearer fragmentation and additional structural information compared to the protonated ions, whose fragments were similar to the CID fragments discussed in part 1 of this study[4].

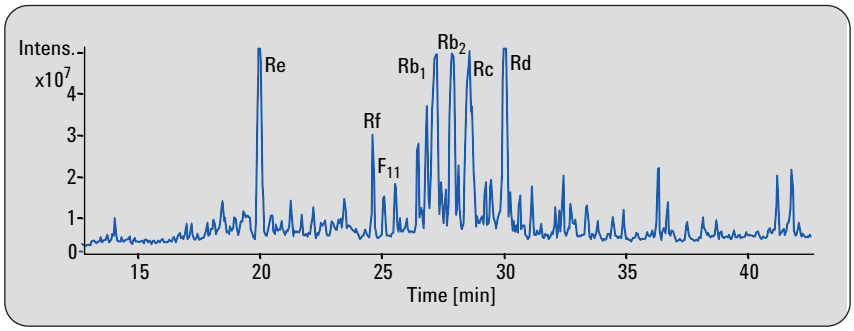

Figure 2
Base peak chromatogram of ginseng extract by high resolution LC ion trap on a 1.8 µm particle column.

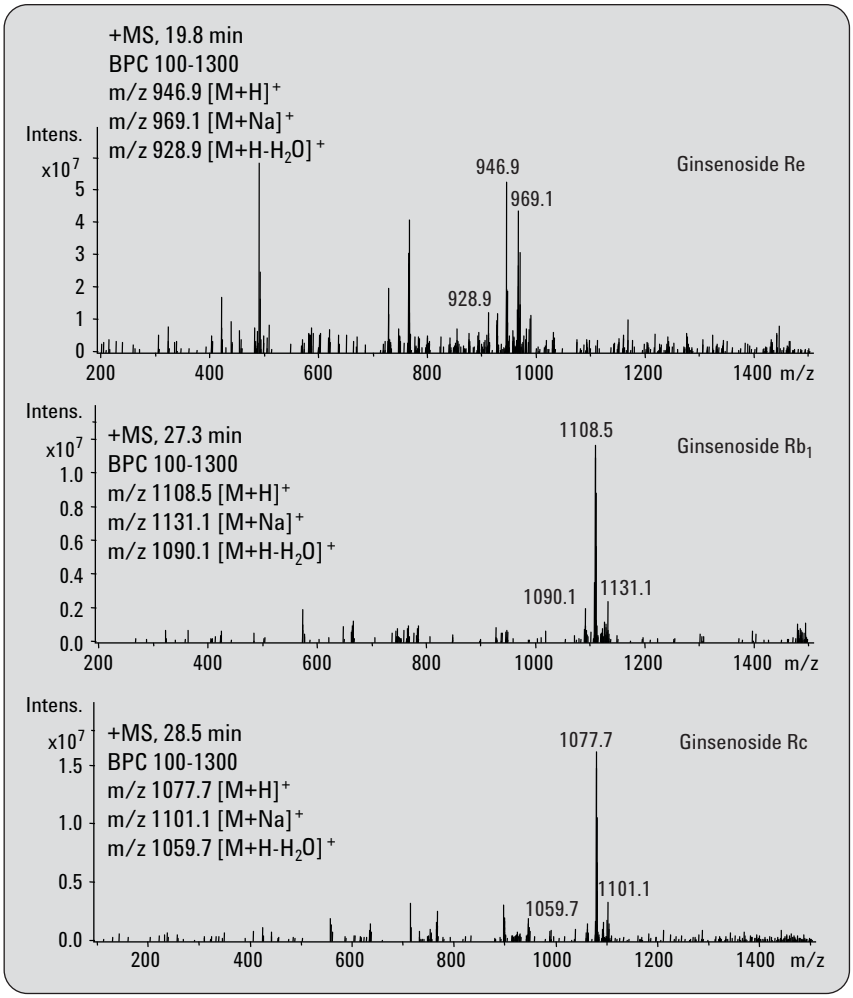

Figure 3
Mass spectra of ginsenosides Re, Rc and Rb_1.

Only one fragment is produced in MS/MS for the ginsenodide Re at m/z 789.9 by a loss of a molecule of glucose from the sodiated molecular ion (figure 4). In MS^3 this sodiated fragment is cleaved into two parts, whereas the detected ion at m/z 349.2 comes from the cleaved disaccharide moiety. In comparison, for the ginsenoside Rc there are two fragments obtained by MS/MS. There is also one at m/z 789.5 and another one at m/z 335.1 for the arabinose saccharide (figure 5). The dissacharide at m/z 365.1 is cleaved off from the ion at m/z 789.5 in the MS^3 stage. The ginsenoside Rb_1 has another different fragmentation pattern where the molecular ion is cleaved to the ions at m/z 789.5 and m/z 365.1 by a loss of the glucose chain in the MS/MS stage. In the MS^3 stage the fragment obtained at m/z 789.5 also loses the same disaccharide at m/z 356.1 (figure 6).

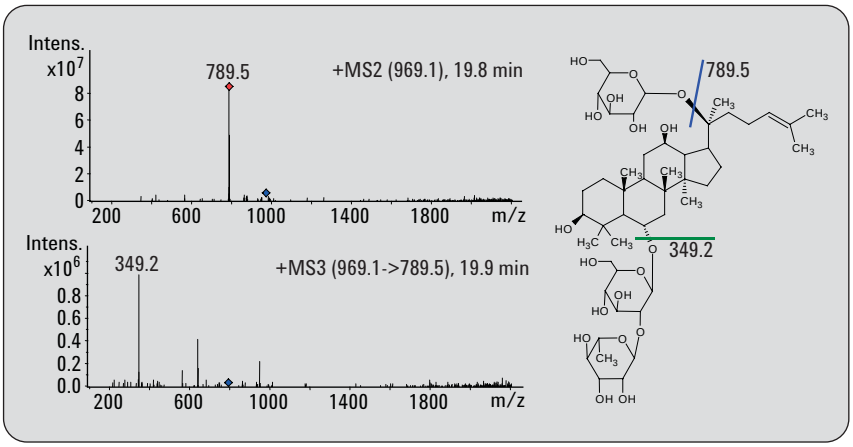

Figure 4
MS/MS and MS^3 fragmentation for structure elucidation of ginsenoside Re with ion trap LC/MS.

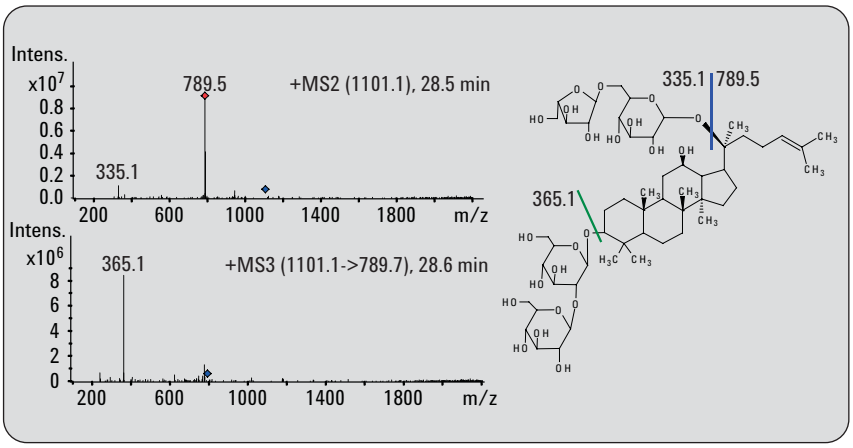

Figure 5
MS/MS and MS^3 fragmentation for structure elucidation of ginsenoside Rc with ion trap LC/MS.

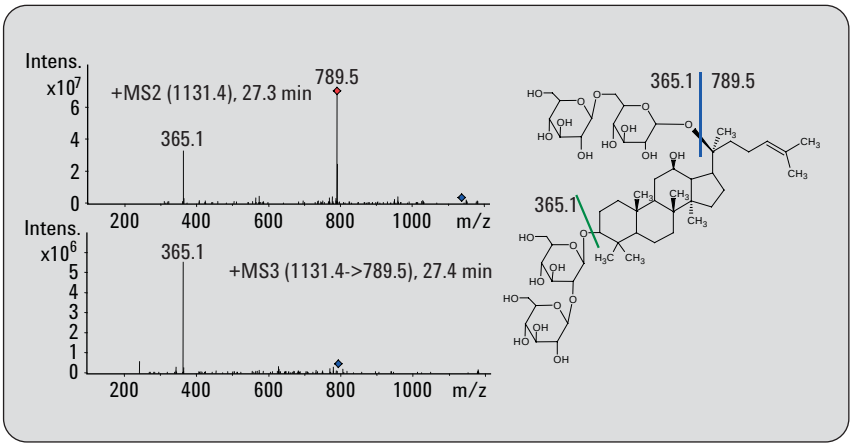

Figure 6
MS/MS and MS^3 fragmentation for structure elucidation of ginsenoside Rb_1 with ion trap LC/MS.

It is possible to distinguish between the different ginsenosides contained in the ginseng root extract with these MS/MS and MS^3 fragmentation patterns because the different saccharide molecules connected to the triterpenoide core structure will be cleaved off in a different but characteristic way. If the specific fragmentation pattern in MS/MS and MS^3 mode of the various ginsenosides is known, it helps to detect the presence of a special compound in the plant extract in a very specific manner with the ion trap MRM. MS/MS-MRM on the sodiated molecular ions of the ginsenosides Re, Rb_1 and Rc shows exactly their presence in the crude ginseng root exact (figure 7). The obtained MS/MS spectra are in accordance with the spectra obtained in the experiments described above (figures 4-6). In addition, the isomeric ginsenosides Rb_2 and Rd are also detected.

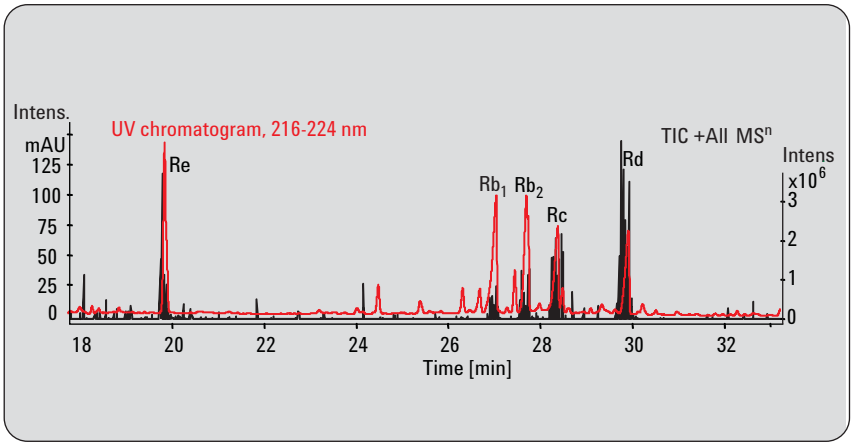

Figure 7
MS/MS-MRM of the sodiated molecular ions of ginsenosides Re, Rb_1 and Rc together with the UV chromatogram obtained from a crude ginseng root.

Conclusion

The Agilent 1200 Series Rapid Resolution LC system in combination with the Agilent 6330 Ion Trap LC/MS proves its capability in structure elucidation of natural products in highly complex extracts from plant origin. In this study a highly complex extract from ginseng root was analyzed using the Agilent 1200 Series Rapid Resolution LC system and the Agilent 6330 Ion Trap LC/MS system. Complex structures of three ginsenosides, which are the main compounds of the extract, could be elucidated by the interpretation of the MS/MS and MS^3 ion trap data obtained. The detailed knowledge of the different fragmentation data can be used to control the quality of natural extracts or pharmaceutical products by ion trap MRM when applied to special ingredients.

References

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