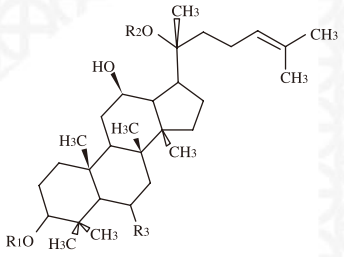
Natural Product Analysis using the LCMS-IT-TOF

Technical Report vol.4

1. Introduction

Recently, the popularity of remedies consisting of natural products found in foods, roots and herbs has increased in both the domestic and global healthcare markets. These compounds, termed “Nutraceuticals”, refer to natural, biologically active chemical species that may be useful in disease prevention or have other additional medicinal properties. As a result of this renewed focus on natural remedies, efficient identification and analysis of active compounds in these products is a growing area of method development. The LCMS-IT-TOF allows researchers in this field to obtain both chemical and structural information by utilizing both the fragmentation power of the ion trap, and the high resolution and mass accuracy of the time-of-flight mass spectrometer.



Ginsenoside	R_1	R_2	R_3
Rb1	-Glc^2-Glc	-Glc^6-Glc	H
Rb2	-Glc^2-Glc	-Glc^6-Ara(p)	H
Rc	-Glc^2-Glc	-Glc^6-Ara(f)	H
Rd	-Glc^2-Glc	-Glc	H
Re	-H	-Glc	-O-Glc^2-Rha
Rg1	-H	-Glc	-O-Glc

Fig. 1 Structure of ginsenosides in forms of 20(S)-protopanaxadiol and -triol.[1]

2. Method

1. The constituents of Panax Quinquefolius, or North American ginseng, were first extracted with hot methanol. The extract was further partitioned with ethyl acetate and water. The aqueous layer was extracted with butanol, and this final extract separated via silica gel chromatography. The elution solvent consisted of a mixture of CH_2Cl_2:MeOH:H_2O in varying ratios from 50:10:1 to 5:5:1.
2. Samples were further separated via RP-LCMS on a Shimadzu Prominence series LC utilizing a Shimadzu Shim-pack VP-ODS column (150 x 2.0 mm; 5.0 μm).
3. Mass spectrometric analysis [(-) ESI] was carried out on a Shimadzu LCMS-IT-TOF (ion trap - time-of-flight hybrid mass spectrometer) with argon gas for ion cooling and CID experiments, and MS^n data were acquired using the “Automatic” mode.
4. Shimadzu’s Formula Predictor Software was also used to verify identification.

3. Instrument Design

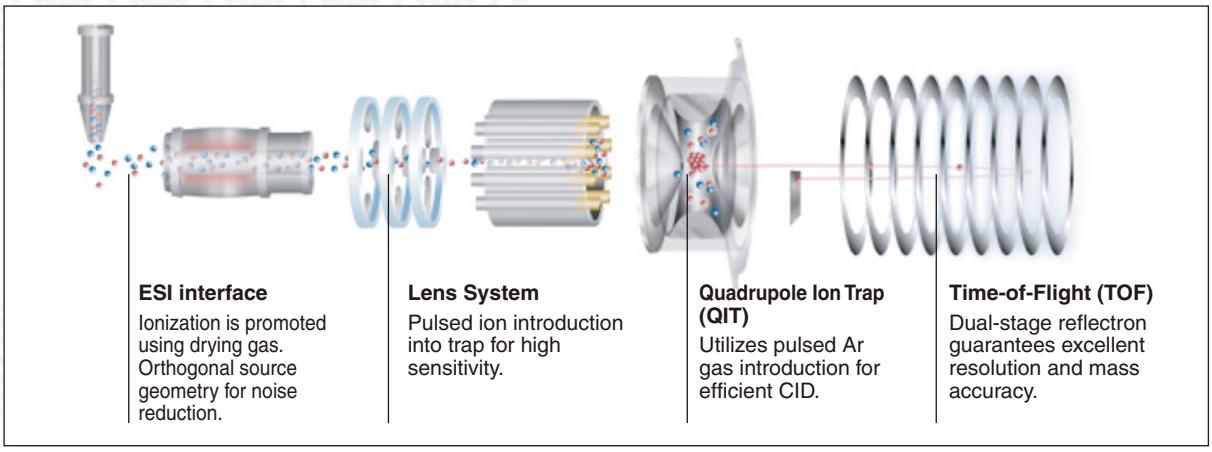


Fig. 2 Schematic representation of the LCMS-IT-TOF

4. Results

Fig. 3 shows the LC-MS chromatograms of North American ginseng. Fractions A - F were collected with varying ratios of extraction solvent CH_2Cl_2:MeOH:H_2O, as indicated in the figure.

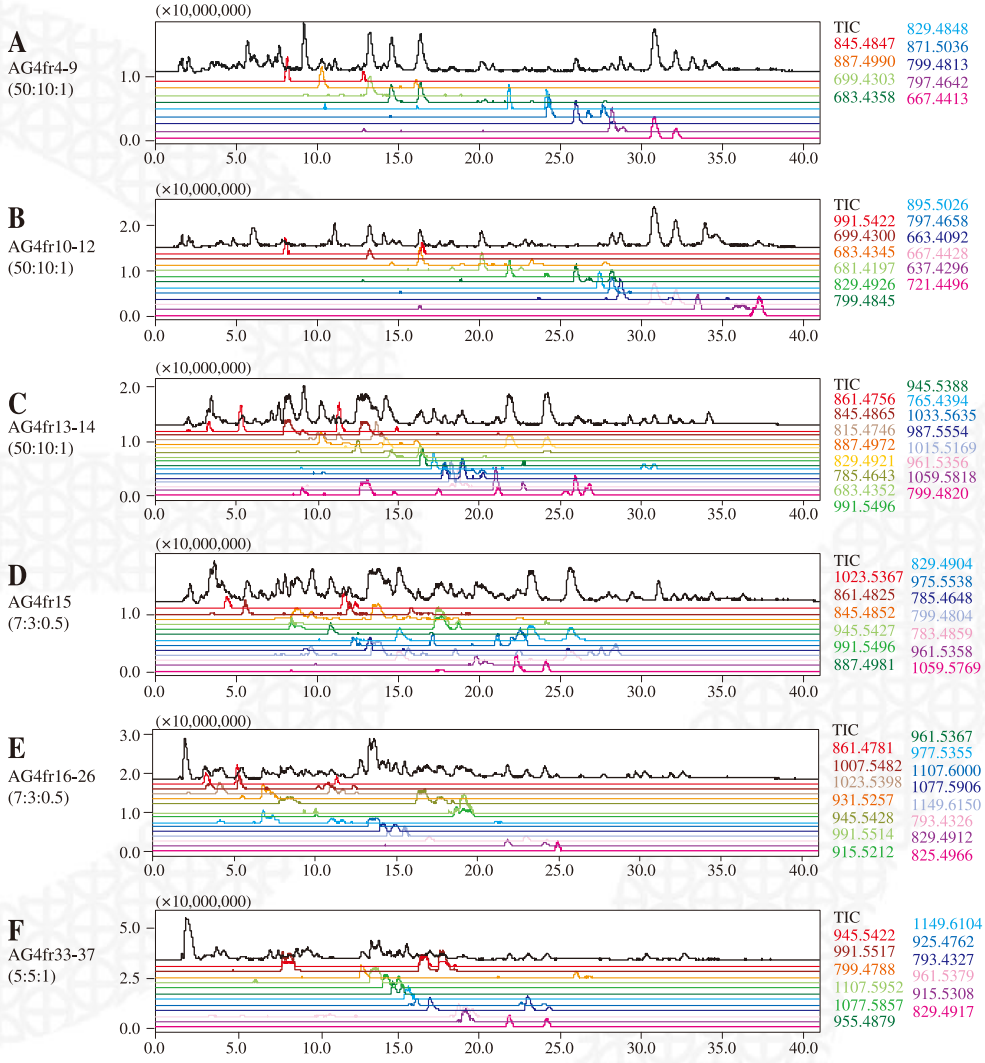


Fig. 3 LC-MS Chromatograms for Fractions of Extracted N. American Ginseng
Fractions were collected with varying ratios of extraction solvent CH_2Cl_2:MeOH:H_2O.

Figures 4 and 5 show the mass spectra when the same m/z 945.54 that eluted at 8 min and 16 min RT was taken as the precursor ion. The observed m/z 459.38 fragment in Fig. 4 is indicative of the protopanaxadiol group found in ginsenoside Rd, and the observed m/z 475.38 fragment in Fig. 5 is indicative of the protopanaxatriol group found in ginsenoside Re.

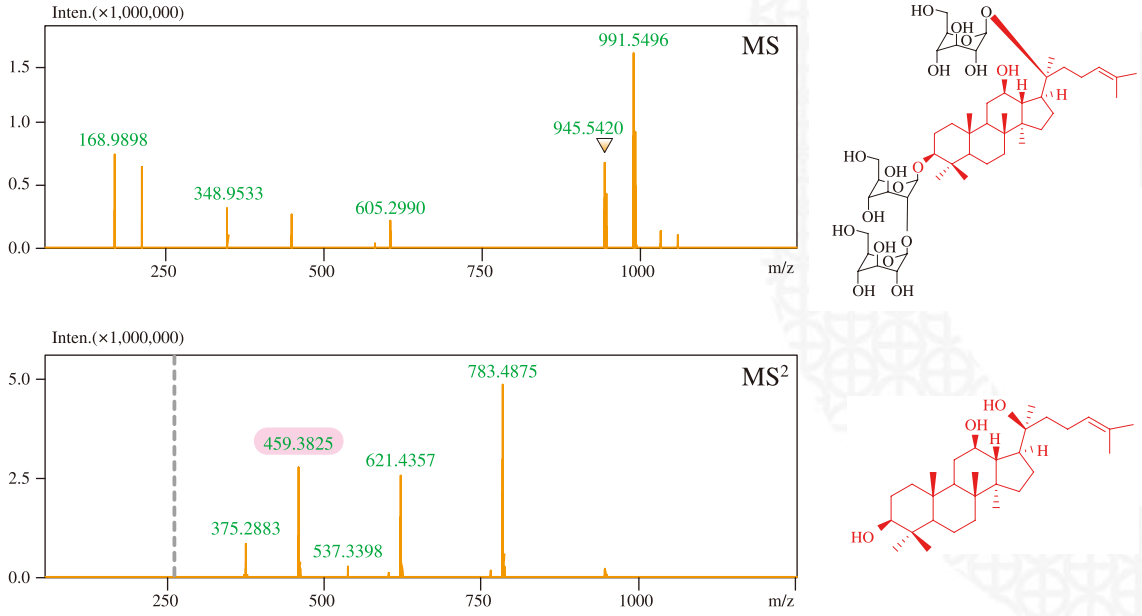


Fig. 4 Mass Spectrum at Retention Time 16 min.
Fragmentation of the 945 m/z ion eluting at RT-16 min gave a characteristic fragment at 459 m/z, indicative of the protopanaxadiol group found in ginsenoside Rd.

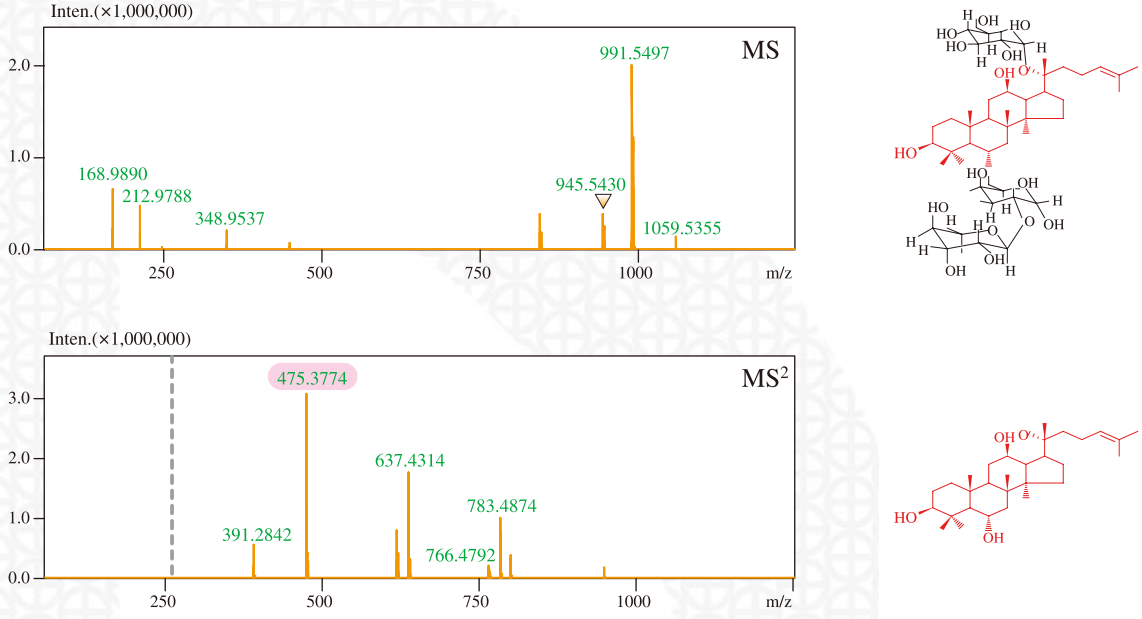


Fig. 5 Mass Spectrum at Retention Time 8 min.
Fragmentation of the 945 m/z ion eluting at RT-8 min gave a characteristic fragment at 475 m/z, indicative of the protopanaxatriol group leading to an assignment of ginsenoside Re.

Figures 6 and 7 show the mass spectra and expected dissociation pathway of ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$). It is shown that dissociation occurs first in the arabinose group and then in subsequent glucose groups.

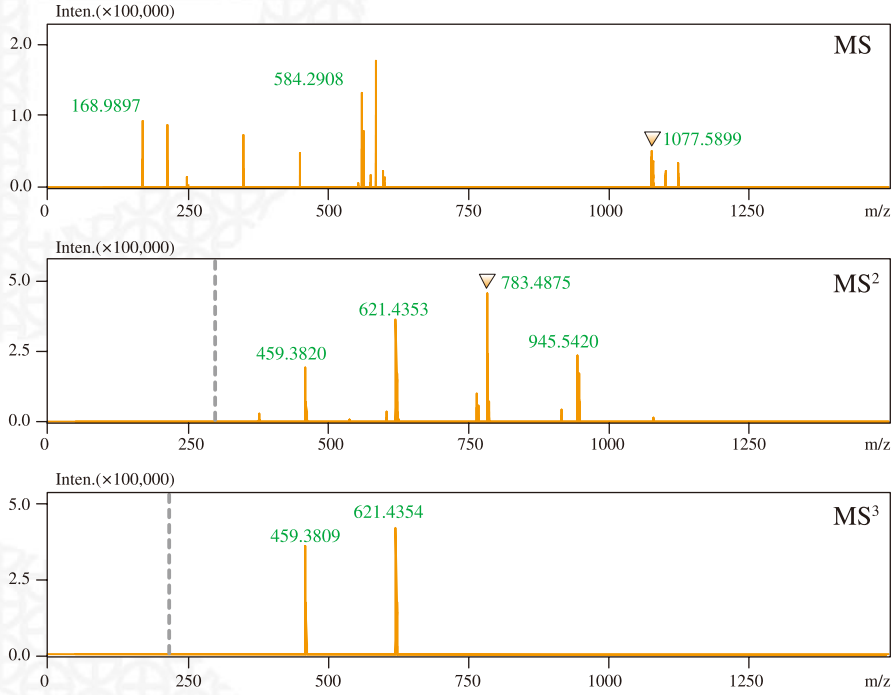


Fig. 6 Mass Spectra for Ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$)
The MS^2 spectrum shows first the loss of the arabinose group and then the subsequent glucose groups (MS^3).

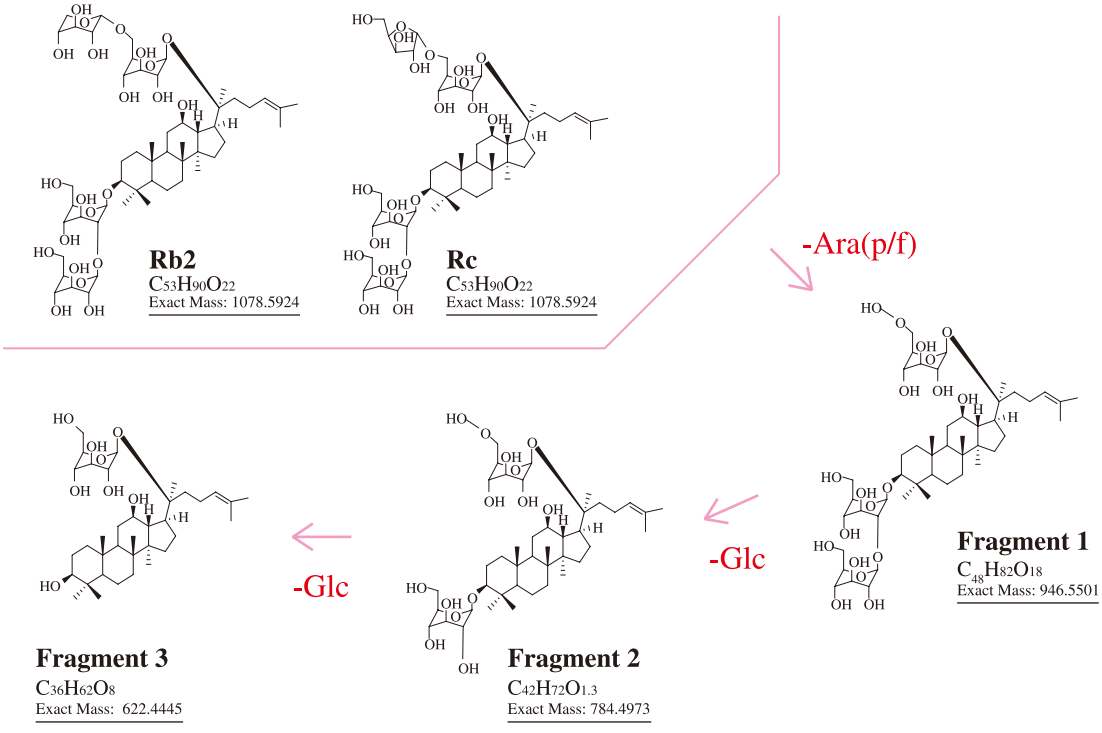


Fig. 7 Dissociation pathway for ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$).

Figure 8 shows the MS to MS^3 spectra of Notoginsenoside R1 ($C_{47}H_{80}O_{18}$). Many constituents showed an adduct of +46 Da which was attributed to the formic acid of the mobile phase.

The formula prediction results for Notoginsenoside R1 are shown in Fig. 9.

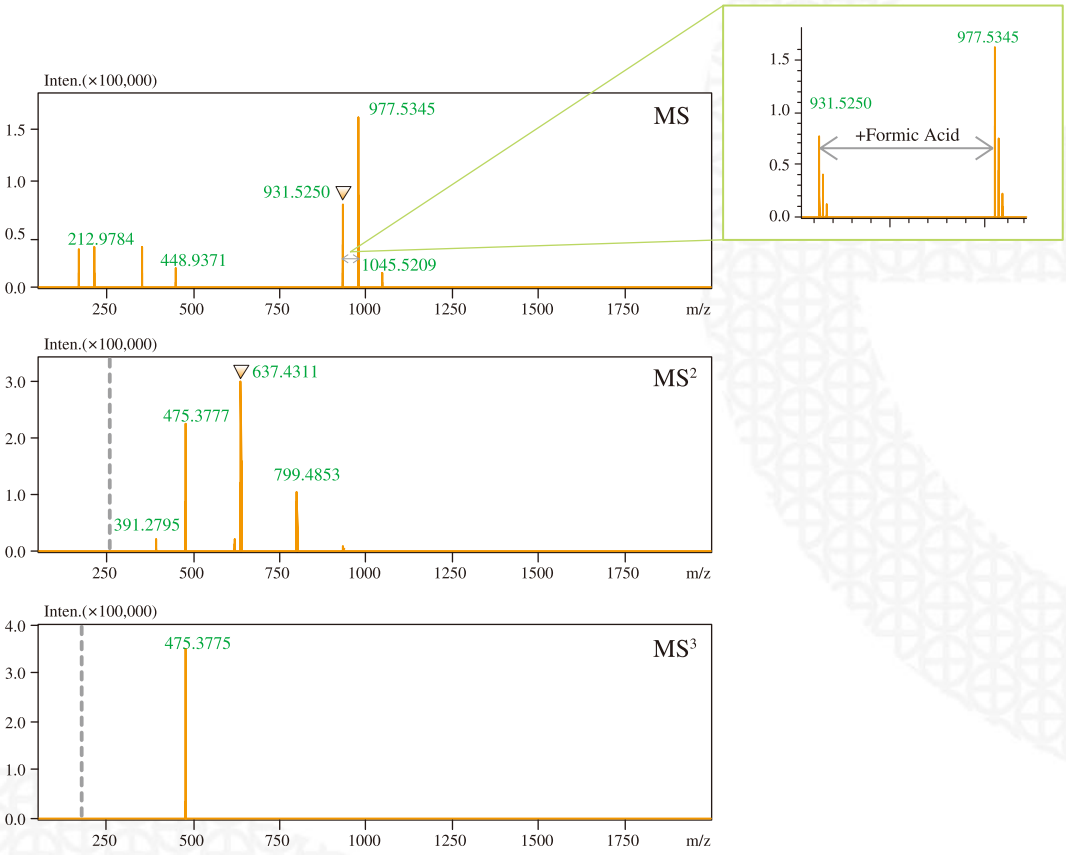


Fig. 8 MS to MS^3 Analysis of Notoginsenoside R1 ($C_{47}H_{80}O_{18}$)

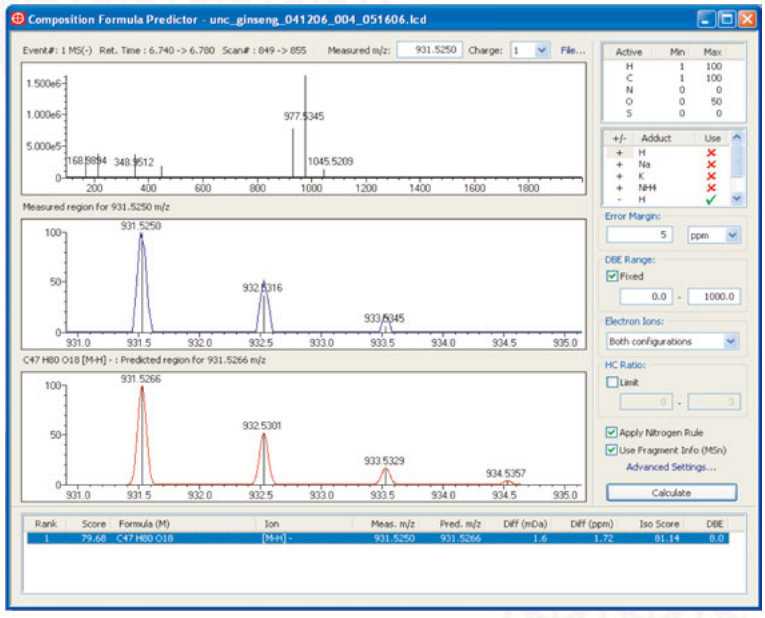


Fig. 9 Formula Prediction Results for Notoginsenoside R1
Formula Predictor results for 931 m/z.

Fig. 10 shows the mass spectra of ginsenoside Rb1. In the MS data, a doubly-charged overlapping dimer appears, but in the MS^2 spectrum, only a singly-charged ion of Rb1 was confirmed. The LCMS-IT-TOF mass accuracy for analysis of ginsenosides is shown in Table 1.

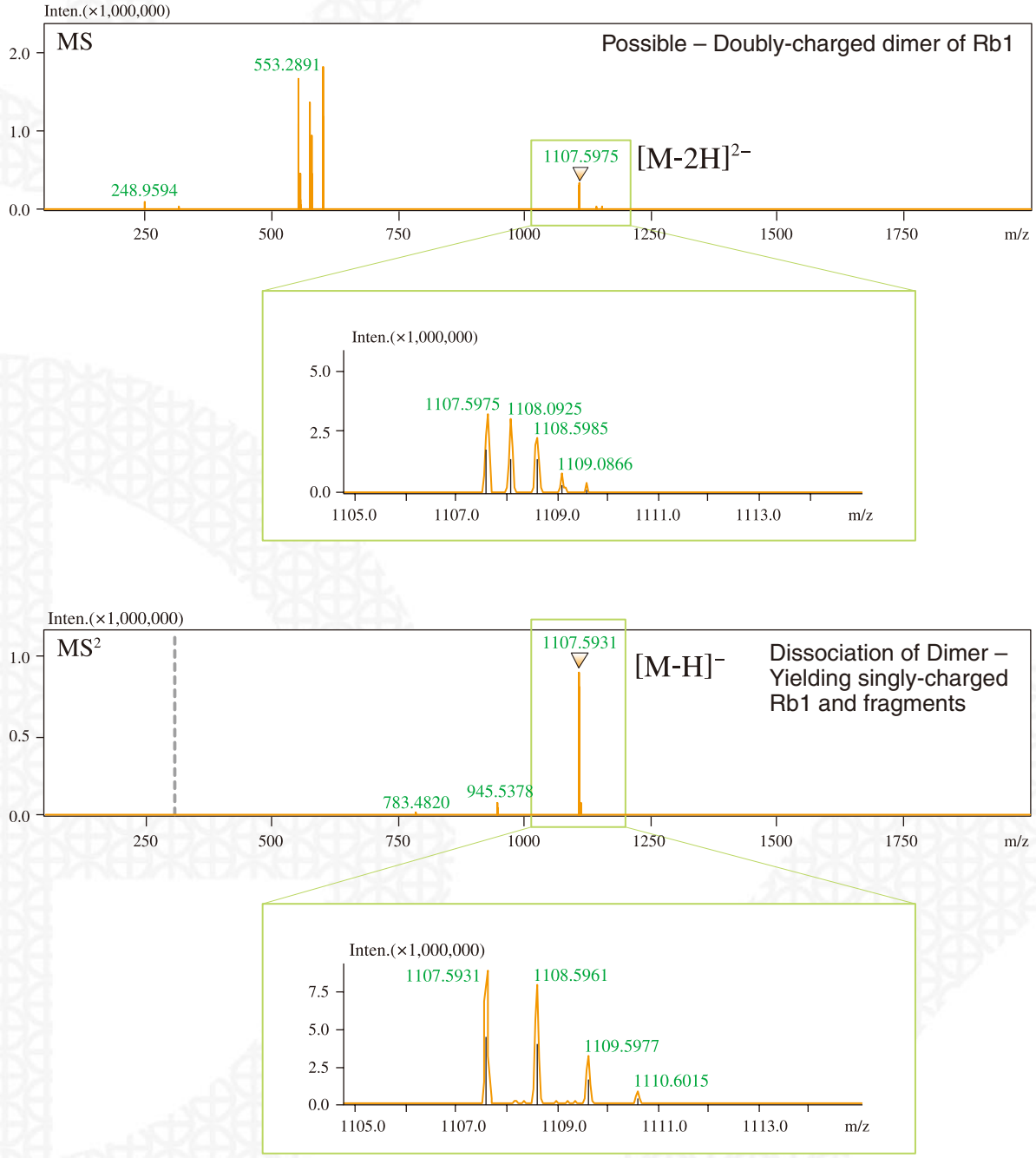


Fig. 10 MS and MS^2 Spectra of Ginsenoside Rb1
Mass spectra showing the dimeric complex of Rb1 and its dissociation after MS^2 experiments.

name	formula [M]	[M-H]-calculated (monoisotopic)	[M-H]-observed (monoisotopic)	mass accuracy (ppm)
Rb1	$C_{54}H_{92}O_{23}$	1107.5951	1107.5979	2.5
Rb2 or Rc	$C_{53}H_{90}O_{22}$	1077.5845	1077.5906	5.6
Rd	$C_{48}H_{82}O_{18}$	945.5423	945.5420	0.3
Re	$C_{48}H_{82}O_{18}$	945.5423	945.5430	0.7
Rd/Re + formic acid	$C_{49}H_{84}O_{20}$	991.5478	991.5496	1.8
Ginsenoside Base	$C_{30}H_{52}O_4$	475.3787	475.3774	2.7*
Ginsenoside Base	$C_{30}H_{52}O_3$	459.3838	459.3825	2.8*
Rg1 + formic acid	$C_{43}H_{74}O_{16}$	845.4899	845.4868	3.7
F11	$C_{42}H_{72}O_{14}$	799.4844	799.4820	3.0
Ro	$C_{48}H_{76}O_{19}$	955.4903	955.4879	2.5
Rg3	$C_{42}H_{72}O_{13}$	783.4895	783.4859	4.6
Rg3 + formic acid	$C_{43}H_{74}O_{15}$	829.4949	829.4921	3.4
Rh1 + formic acid	$C_{37}H_{64}O_{11}$	683.4370	683.4352	2.6
Rh2 + formic acid	$C_{37}H_{64}O_{10}$	667.4421	667.4419	0.3
F1	$C_{36}H_{62}O_9$	637.4316	637.4296	3.1
Rs3	$C_{44}H_{74}O_{14}$	825.5000	825.4966	4.1
Notoginsenoside R1	$C_{47}H_{80}O_{18}$	931.5266	931.5250	1.7

* Mass accuracy of MS^2 spectra

Table 1 Mass Accuracy Data for the Analysis of Ginsenosides using the LCMS-IT-TOF

5. Conclusions

1. Ginsenosides from American ginseng were successfully separated and analyzed using Shimadzu's LCMS-IT-TOF (ion trap - time-of-flight hybrid mass spectrometer). Adducts with formic acid and dimeric complexes were observed.
2. Structural information and mass accuracy data can be obtained in a single experiment.
3. Mass accuracy was routinely below 5 ppm for the analysis utilizing a simple auto-tuning prior to the start of the experiments (approx. 30 minutes)
4. Fragmentation data successfully led to the correct assignment of ginsenosides with similar chemical formulae.
5. Shimadzu's Formula Predictor Software utilizes both mass accuracy and fragmentation information from MS^n experiments to aid researchers in determining the composition of unknowns.

References:

[1]Wang, X.; Sakuma, T.; Asafu-Adaje, E.; Shu, G.K. Anal. Chem.1999, 71, 1579-1584.
[2]Taniguchi, J.; Kawatoh, E.; Itoi, H.; Bilsborough, S.; Loftus, N.; Miseki, K. Proc. 52nd ASMS Conf. Mass Spectrom. and Allied Topics. Nashville, TN, 2004.
[3]Fuzzati, N. J. Chromatogr. B2004, 812, 119-133.

LCMS-IT-TOF LIQUID CHROMATOGRAPH MASS SPECTROMETER

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