Natural Product Analysis Utilizing an Ion Trap – Time-of-Flight Mass Spectrometer (IT-TOF)

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Overview

Purpose :
Analysis of the ginsenoside constituents of American ginseng

Methods :
Solvent Extraction of raw American ginseng and further separation with silica-gel chromatography
LC-MS/MS analysis on a LCMS-IT-TOF allowing for MS^n fragmentation and mass accuracy

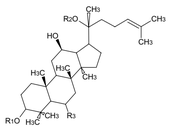
Results :
Dimeric species of ginsenosides were observed.
Fragmentation patterns lead to unique identifications for isomeric ginsenosides.
14 known ginsenoside species were identified from 6 fractions within a mass accuracy of 5 ppm or less.

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. B 2004, 812, 119-133.

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 the 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 as it utilizes both the fragmentation power of the ion trap and the high resolution, and mass accuracy, of a 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

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

Methods

- The constituents of Panax quinquefolius or American ginseng were first extracted with hot methanol. The extract was further partitioned with ethyl acetate and water. The water layer was extracted with butanol, and this final extract separated via silica gel chromatography. Elution solvent included a mixture of CH_2Cl_2:MeOH:H_2O from a ratio of 50:10:1 – 5:5:1.
- Samples were further separated via RP-LCMS on a Shimadzu Prominence series LC utilizing a Shimadzu Shim-pack VP-ODS column (150 × 2.0 mm ; 5.0 µm).
- Mass spectrometric analysis [(-) ESI] was carried out on a Shimadzu LCMS-IT-TOF with argon gas for ion cooling and CID experiments. MS^n data was acquired using the "Automatic" mode or data-dependent function.
- Shimadzu's Composition Formula Predictor was also used to verify identifications.

Instrumental Design[2]

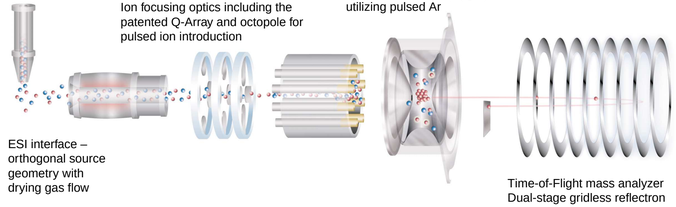


Figure 2. Schematic representation of the LCMS-IT-TOF

Results

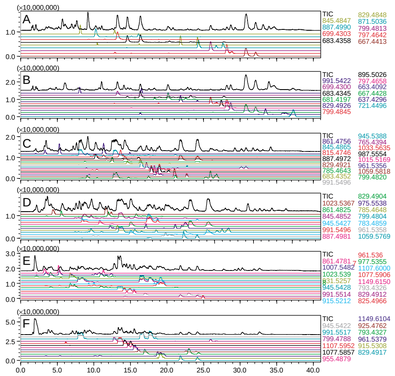


Figure 3. LC-MS chromatograms for fractions of extracted American ginseng. Fractions were collected with varying ratios of extraction solvent CH_2Cl_2:MeOH:H_2O. A – AG4fr4-9 (50:10:1); B – AG4fr10-12 (50:10:1); C – AG4fr13-14 (50:10:1); D – AG4fr15 (7:3:0.5); E – AG4fr16-26 (7:3:0.5); F – AG4fr33-37 (5:5:1).

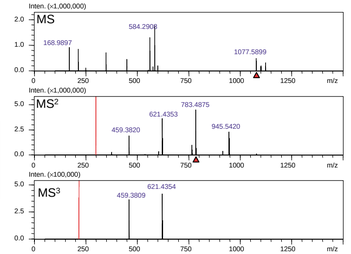


Figure 6. Mass spectra for ginsenoside Rb2 or Rc ($C_{53}H_{90}O_{22}$). The MS^2 spectrum shows first the loss of the arabinose group and then subsequent glucose groups (MS^3).

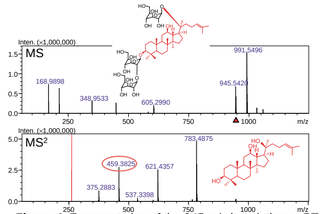


Figure 4. Fragmentation of the 945 m/z ion eluting at RT ~16 min gave a characteristic fragment at 459 m/z indicative of the protopanaxadiol group found in ginsenoside Rd.

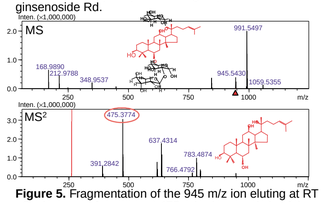


Figure 5. Fragmentation of the 945 m/z ion eluting at RT ~8 min gave a fragment at 475 m/z characteristic of the protopanaxatriol group leading to an assignment of ginsenoside Re.

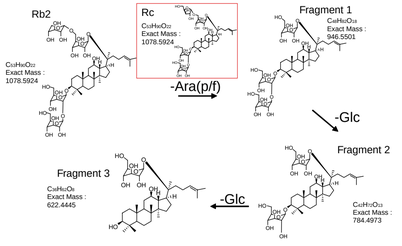


Figure 7. Dissociation pathway for ginsenoside Rb2 or Rc.

Results

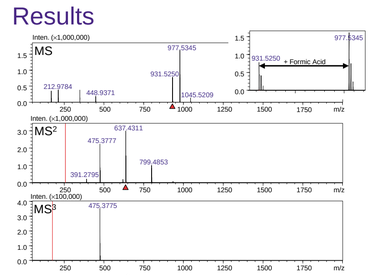


Figure 8. MS – MS^3 analysis of Notoginsenoside R1 ($C_{47}H_{80}O_{18}$). Many constituents showed an adduct of + 46 Da which was attributed to the formic acid of the mobile phase.

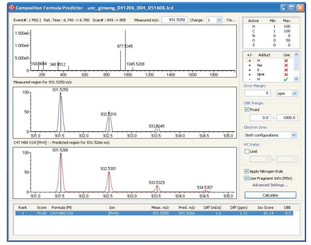


Figure 9. Composition Formula Predictor results for 931 m/z.

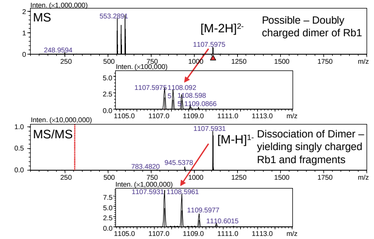


Figure 10. Mass spectra showing the dimeric complex of Rb1 and its dissociation after MS/MS experiments.

Table 1. Mass accuracy data for the analysis of ginsenosides on the LCMS-IT-TOF.

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	$C_{43}H_{72}O_{16}$	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

* MS/MS mass accuracy

Discussion and Conclusions

- Ginsenosides from American Ginseng were successfully separated and analyzed using Shimadzu's LCMS-IT-TOF. Adducts with formic acid and dimeric complexes were observed.
- Within one experiment, structural information and mass accuracy data can be obtained.
- Mass accuracy was routinely below 5 ppm for the analysis utilizing a simple auto-tuning prior to the start of experiments (~ 30 min).
- Fragmentation data successfully lead to the correct assignment of ginsenosides with similar chemical formulae.
- Shimadzu's Composition Formula Predictor Software utilizes both mass accuracy and fragmentation information from MS^n experiments to aid researchers in determining the composition of unknowns.