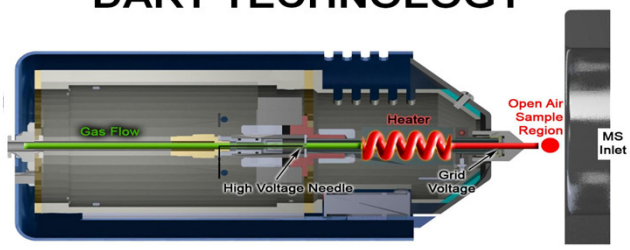
Determination of Ginsenosides in Ginseng Roots and Commercial Products by using In-situ Derivatization Direct Analysis in Real Time

Rachel N Liu[1]; Hao Yue[2]; Jordan Krechmer[3]; Lili Jiao[2]; Charles C Liu [1];
Brian Musselman[3]; Shuying Liu[2]
[2]Jilin Ginseng Academy, Changchun University of TCM, Changchun, China;
[3]IonSense, Inc., Saugus, MA, USA, [1]ASPEC Technologies, Beijing, China

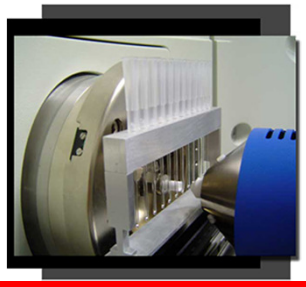
Abstract

Determination of Ginsenosides in roots is often completed by time consuming LC/MS based method. The rational for these test is to permit characterization of the distribution and content of mono- di- and tetra-glycosides in the raw material or processed products in order to assess quality and potency of traditional medicines. We have developed a rapid test that produces a stable permethylated ginsenosides seconds after exposure of either raw materials or finished products to a heated gas stream containing metastable atoms of helium. The utility of this method for rapid sampling of materials in support of quality control efforts and production improvement methods will be discussed.

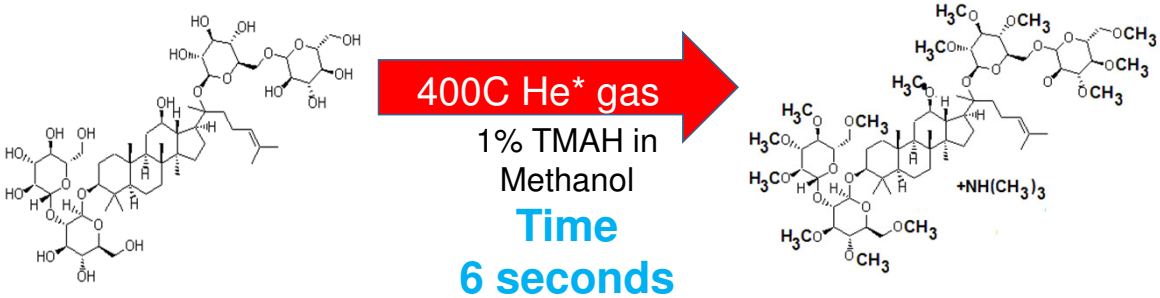
DART TECHNOLOGY



Corona Discharge generates helium metastable atoms which are heated, stripped of ions, and directed out the end of the source at the sample positioned near the API



DART with 12 Samples mounted on the robotic arm in front of the API inlet of a LCQ DECA LC/MS

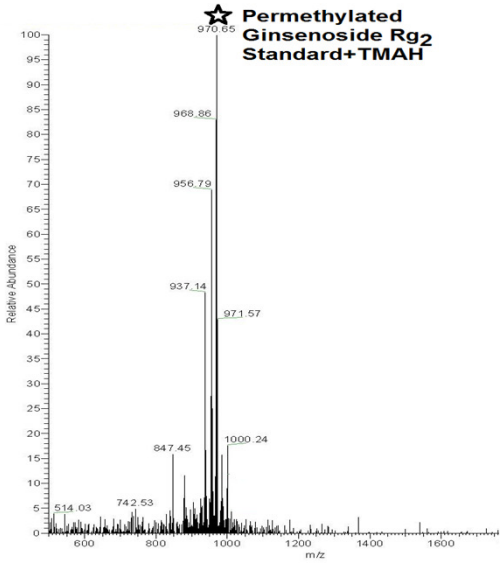


Method

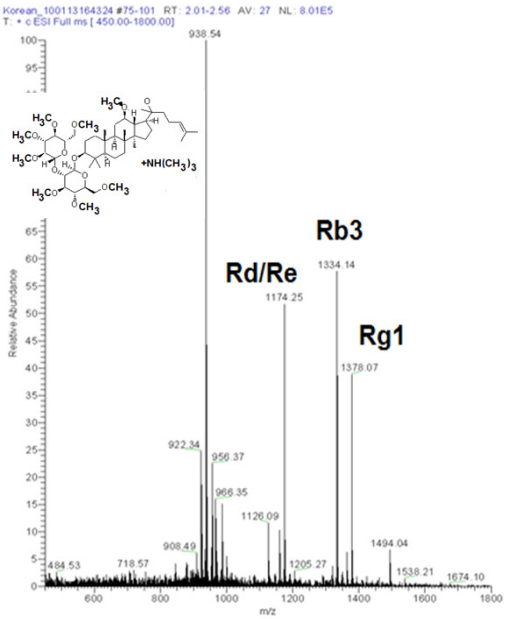
Ginsenoside standards and commercial products were purchased for analysis. The closed end of a glass capillary melting point tube was dipped into the standard so that a small fraction of each was observed to be on the tube surface. When analyzed directly by using DART with helium gas heated to low, medium and high temperatures of 150C, 250C, and 350C respectively, no ions with mass values in the expect range were detected. The standards were once again sampled with the glass melting point tube, however to facilitate the formation of a more thermally stable derivatization a 2ul aliquot of 1% Tetramethyl-ammonium hydroxide (TMAH) in methanol was pipetted onto the tube which was then immediately positioned in the heated gas stream coming from the DART source. The in-situ derivatization reaction facilitates methylation of the carbohydrates and free alcohol groups on the core steroid. The reaction scheme is shown in above.

Ginsenoside Rb2 Standard

A small percentage of base in the presence of methanol in the gas phase leads to methylation of the alcohols.



Analysis of Powders is ideal since no water is present. Isomers are not differentiated, however major ginsenosides are detectable shown below

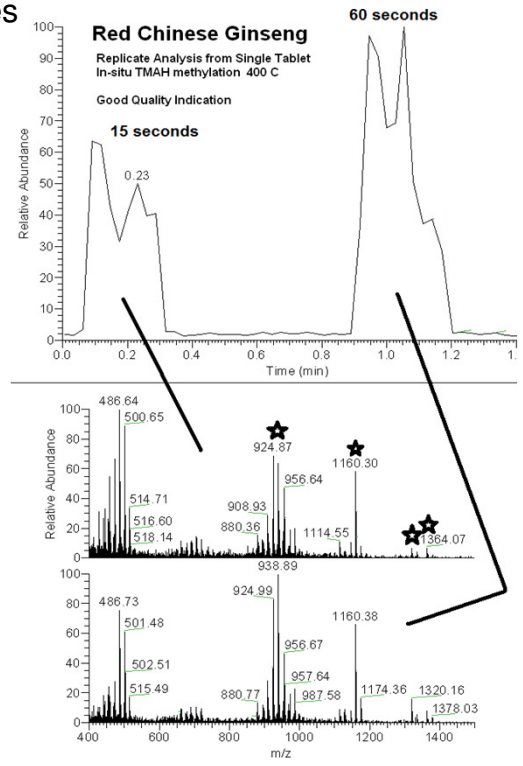


Detection of the intact ginsenosides and the major fragment at 938 dalton from loss of the disaccharide provide a mass spectrum that characterizes the root.

Saponins Detected after derivatization

From "Ginsengs A Comparative Analysis" Central Research Institute, Seoul Korea

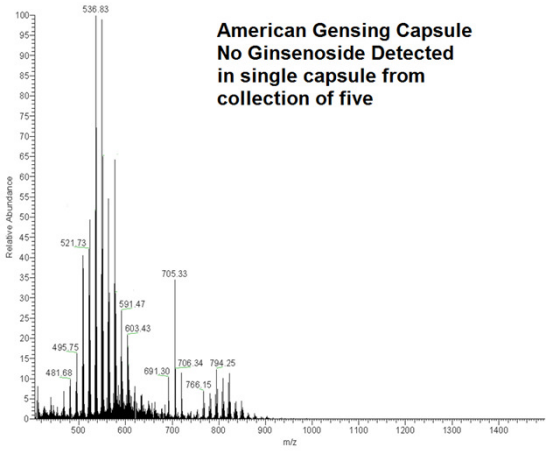
Saponin	Korean White Ginseng	Korean Red Ginseng	American Gensing	Mass Values of Permethyl-TMAH derivatives detected
G-Ra$_1$	0.03	0.02	-	-
G-Ra$_2$	0.02	0.03	-	-
G-Ra$_3$	0.005	0.005	-	-
G-Rb$_1$	0.5	0.4	1.8	1378
G-Rb$_2$	0.2	0.2	0.03	1334
G-Rb$_3$	0.05	0.014	0.03	1034
G-Rc	0.3	0.1	0.3	1078
G-Rd	0.2	0.036	0.5	1174
MG-Rb$_1$	0.8	-	-	-
MG-Rb$_2$	0.4	-	-	-
MG-Rc	0.3	-	-	-
MG-Rd	0.1	-	-	-
G-Rg$_3$	0.0003	0.029	-	970
G-Rh$_2$	-	0.001	-	766
G-F$_2$	-	-	0.018	-
G-Rs$_1$	-	0.008	-	-
G-Rs$_2$	-	0.01	-	-
G-R$_1$	0.002	0.015	0.01	-
Gy-	-	-	0.03	-
N-R$_4$	-	0.002	-	-
Saponin in triol				
G-Re	0.2	0.2	1	1174
G-Rf	0.05	0.066	-	-
20Glc-Rf	0.005	0.008	-	-
G-Rg$_1$	0.2	0.3	0.2	1000
G-Rg$_2$	0.014	0.034	0.008	970
G-Rh$_1$	0.0015	0.013	-	796
N-R$_1$	0.002	0.007	-	-

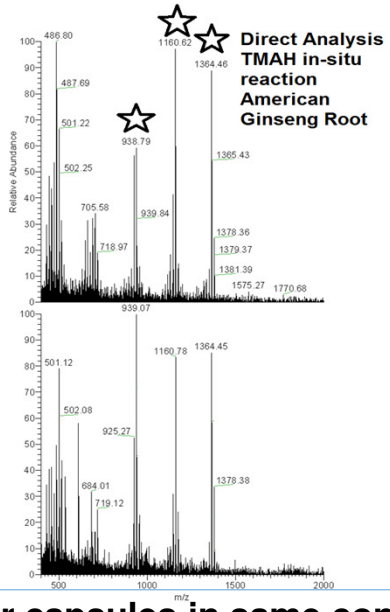


Sample preparation is minimal. The DART driven reaction occurs rapidly. In a non-automated configuration sample analysis is complete in less than 1 minute

Quality Assessment in Second per Sample

Bottle of American Ginseng with 100 tablets

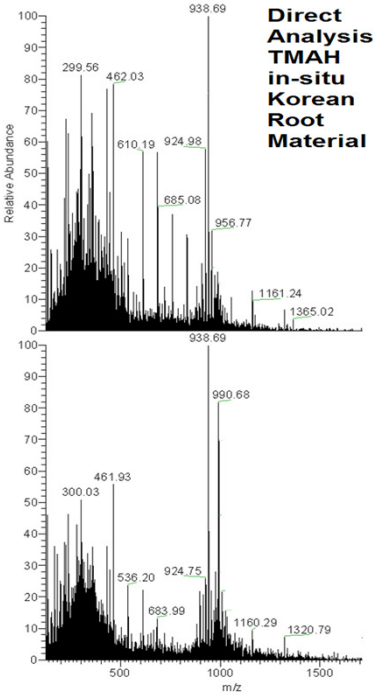


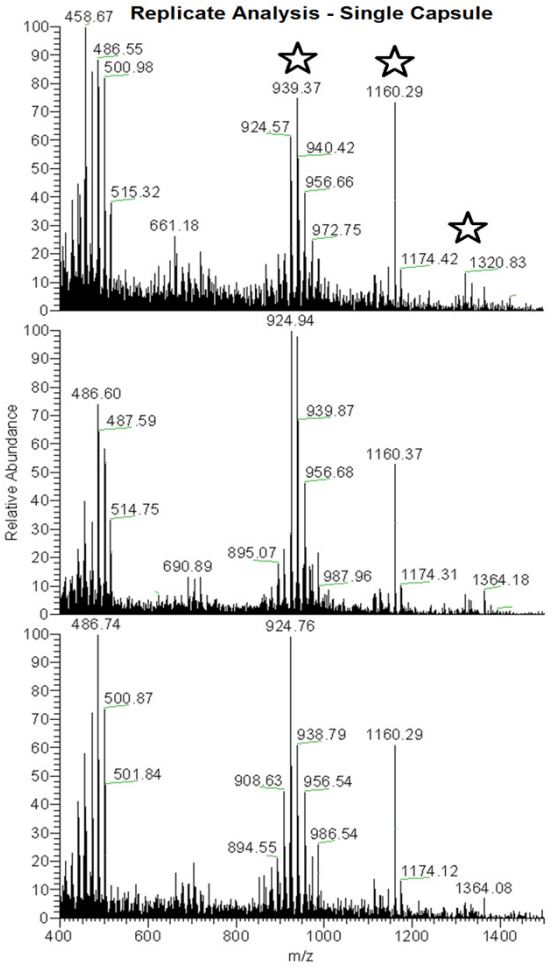


American Ginseng with no ginsenosides detected in powder content of capsule

Other capsules in same container contain ginsenosides typical for the product (top).

Korean Ginseng product with low abundance of larger ginsenosides detected in capsule

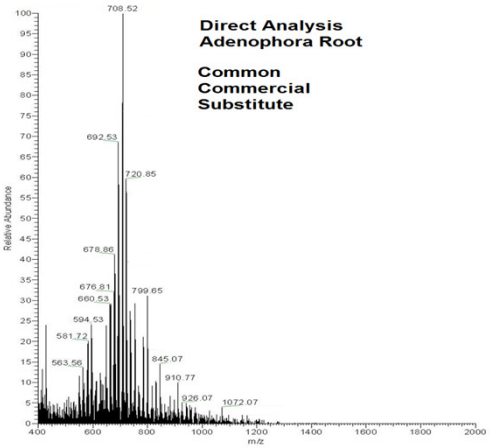


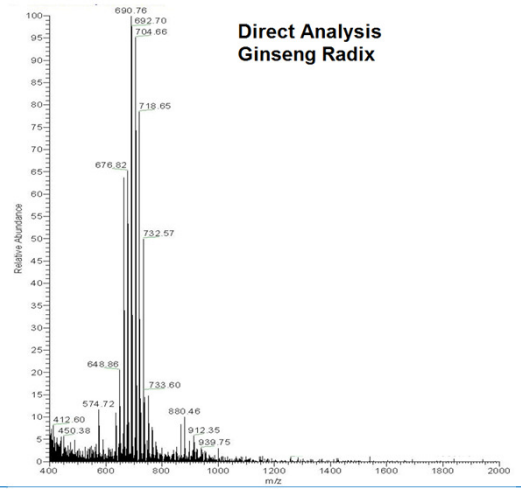


Multiple analyses from same capsules demonstrate reproduciblity

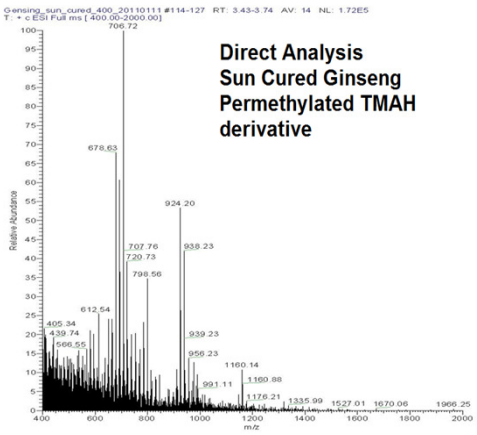
Analysis of Commercial Ginseng Products

Adenophora and Gensing Radix material analyzed are common substitutes which appear to lack larger more complex saponins

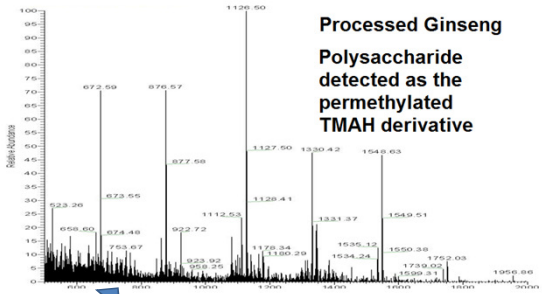


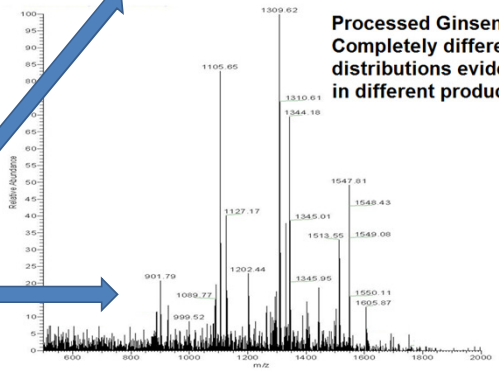


Traditional product determined to contain either polysaccharides or more complex, larger molecules the structure of which have not been determined



Possibilities for process monitoring are evident with the rapid detection of changes in ginsenoside distribution after sun drying, a common practice in production of traditional medicines (above)





Conclusions:

- Rapid DART-based determination of saponins is facilitated by using an in-situ derivatization scheme
- Reaction products yield ions characteristic of a permethylated saponin with one tetramethyl ammonium group reacted with an alcohol.
- Spectra from ion trap and TOF instruments may vary from one another with ion trap units often producing an incomplete reaction product
- Spectra can be collected in a few seconds per sample
- Analysis of representative materials yield results that is consistent
- with the literature based on distribution and type of ginsenoside
- Isomers are not determined using the method