Role of AB SCIEX Mass Spectrometers in Ginseng R & D

[1]Tom K. Moy, [2]Ed Lui, [1]Robert I. Ellis, [1]Carmai Seto, [1]Xu Guo, [1]Eva Duchoslav, [1]YunYun Zou, [1]Suma Ramagiri, [3]Byung-Hee Shin, [3]Sun-Hoon Yeo, [3]Daeseung Lee and [1]Takeo Sakuma
[1] AB SCIEX Concord, ON.; [2]Ontario Ginseng Innovation & Research Consortium, The University of Western Ontario, London, ON; [3]AB SCIEX (Korea), Seoul, Korea

AB SCIEX and Ginseng R & D

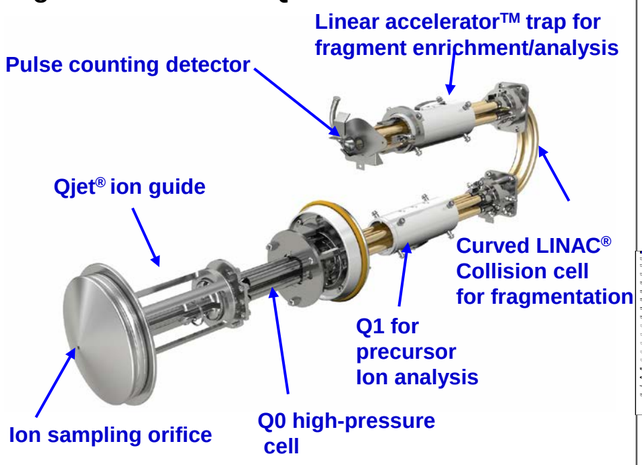
Since mid-1970's Southern Ontario-based SCIEX Inc., predecessor of AB SCIEX, has been providing highly sophisticated and sensitive analytical tools, called "mass spectrometers" (MS) to the pharmaceutical, chemical, biotechnology, food-and-beverage industries; clinical, forensic, environmental and academic communities world-wide. We have a global sales/marketing/support network and offer solutions to various analytical challenges. This presentation introduces how MS can be utilized to develop a market for Ontario-grown ginseng products.

More than 220 growers over 6,000 acres of cultivation and 4 million pounds of harvest in 2004 belonging to the Ontario ginseng Growers Association (OGGA) have the largest production of North American ginseng (*Panax quinquefolius*) in the World. Ginseng production is increasing, as the government's support of tobacco has been declining. In order to help this important Ontario industry, we are currently collaborating with Ontario Ginseng Innovation - Canadian Institute of Chinese Medicinal Research (OGIRC-CICMR) for profiling ginseng grown in several geographic areas; for screening toxic chemical residues in commercial ginseng products and examining health benefits and metabolism of Ginseng.

MASS SPECTROMETERS (MS)

MS analyzes various chemicals by means of m/z (mass-to-charge ratios). Most natural compounds consist of carbon, hydrogen, oxygen, nitrogen and occasional sulphur, phosphorus and metals. For instance, ginsenoside, Rb1, most abundant active ingredient in American ginseng, consists of 54 carbon, 92 hydrogen and 23 oxygen atoms with a mono-isotopic mass of 1,108.60294 Dalton. In constrast, ginsenoside Rf, Rg1, Rg2 are abundant in Chinese/Korean ginseng (Panax ginseng). Rg1 consists of 42 carbon, 72 hydrogen and 14 oxygen atoms with a mono-isotopic mass of 800.49221 Dalton. These compounds is ionized in solution under the influence of an electric field at ambient pressure. We introduce these ions into a vacuum chamber where we have a series of ion lenses. Ions are separated by m/z under the influence of combined radio-frequency/direct current field (rf/dc), or by high potential electric field. AB SCIEX design and manufacture several types of mass spectrometers, but in our current collaboration, we use the QTRAP® 5500 hybrid linear ion trap – triple quadruple (LIT-QQQ) for fast metabolism work and the tripleTOF™ 5600 systems for detailed profiling of signature compounds and elucidation of metabolic fates.

Fig.1 : Schematic of QTRAP® 5500



Precursor ions generated at ambient pressure are sampled through the ion sampling orifice where neutral species are swept away with nitrogen curtain gas. The ion guide and Q0 further enriches ions and send ions to Q1, where precursor ions are separated by m/z. Selected precursor ions enter the curved LINAC collision cell (Q2) where they collide with nitrogen molecules to form unique fragments. Fragments are m/z analyzed by the linear accelerator trap, and detected by pulsed counting detector. The digital signal is fed to a computer that controls the mass spectrometer and a liquid chromatograph, a common separation tool. The system is equipped with a number of patented technologies and suited for fast screening and quantification of targeted compounds utilizing fast scan speed and positive/negative ion mode polarity switching for many ion species. A linear Q2 version, 3200QTRAP® is routinely used as walk-up system by food inspectors to screen foods for the presence of over 200 agrochemicals, mycotoxins and other toxic matters that may affect our health.

Fig. 2: Spiked mycotoxins in ginseng root

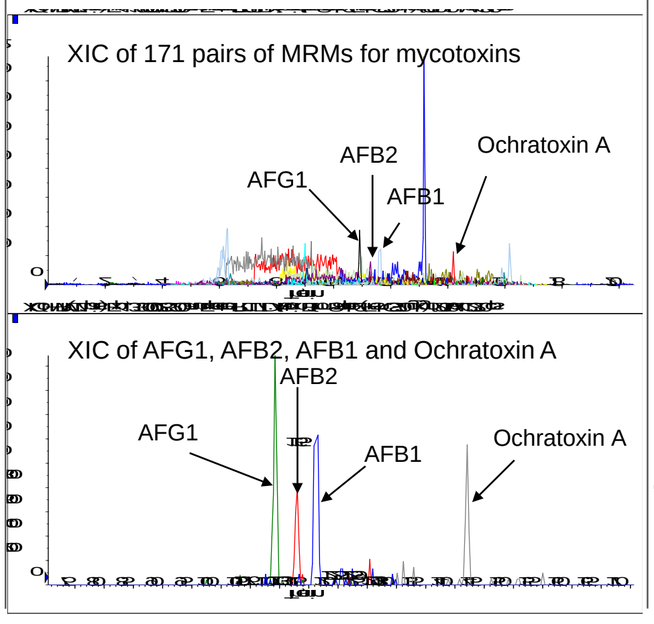


Fig. 2 is an example of mycotoxin (e.g., aflatoxins) screening using a 3200 QTRAP®. Fungal growth can produce mycotoxins during cultivation, storage, transportation or distribution. Potentially harmful mycotoxins were spiked into clean ginseng, and analyzed to demonstrate the system's suitability for routine inspection.

Fig. 3: Screening for 247 agrochemicals

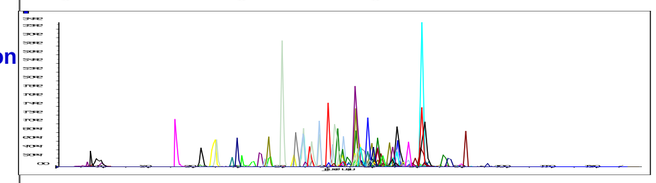
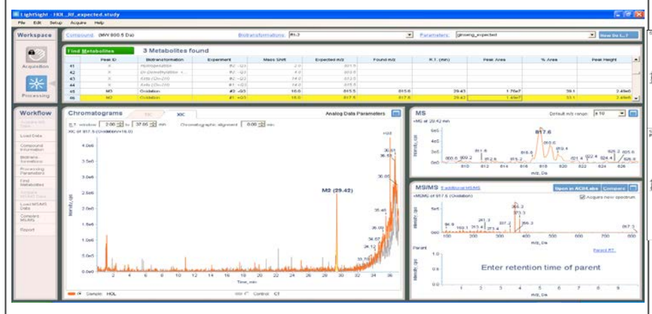


Fig. 3 is an example of output from a mixture of 247 agrochemicals. Two ion pairs are used for each chemical species, first ion pair for quantification, and the second ion pair for confirmation. A total of 494 ion pairs are monitored using our scheduled MRM method in order to produce high quality data. Detection limits are in the 1 – 10 µg/kg range.

Fig. 4: Metabolite ID with QTRAP® 5500



Zucker rats (model for obesity) were dosed daily with Ontario ginseng for 42 days to evaluate the effect on vascular and reproductive health. Plasma was obtained to analyze for ginsenosides and their metabolites to obtain the pharmacokinetic profile. Since the rats were dosed with ginseng extracts, metabolite identification becomes very complex. There are dozens of possible "parent drugs." Of the *ca*. 30 different ginsenosides, 6 (Rg1, Re, Rb1, Rc, Rb2 and Rd) account for over 90% of the ginsenoside content in the root which reduces the complexity of the analysis. Manually interpreting all this data, even when focusing on 6 ginsenosides is extremely time-consuming; the processing time was significantly reduced using automated metabolite identification software, LightSight® to come up with the following metabolite structures.

Fig. 5: Proposed structures of metabolits in rat plasma

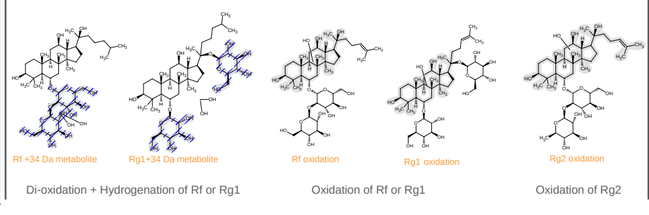
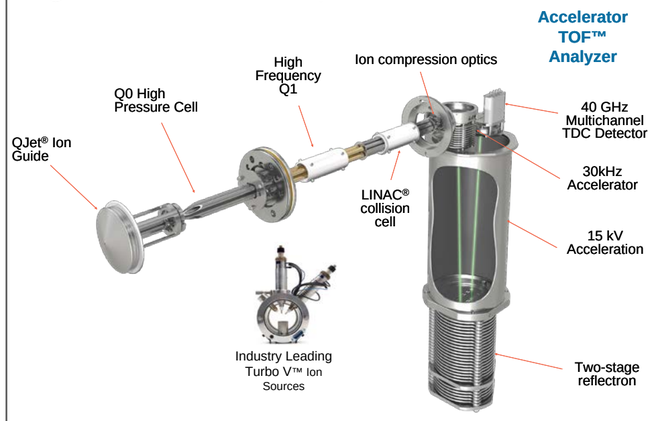


Fig.6 : Schematic of TripleTOF™ 5600



The QTRAP® systems offer unit mass resolution. In contrast, our new TripleTOF™ 5600 is equipped with a time-of-flight tube enabling a high mass resolution of ≥ 43,000, with ≤1 ppm mass accuracy over 30 min. It is suited for the analysis of isobaric compounds (same nominal mass but different elemental compositions) present in a complex sample such as ginseng.

Fig. 7 :TOFMS and MS/MS spectra of Rg1

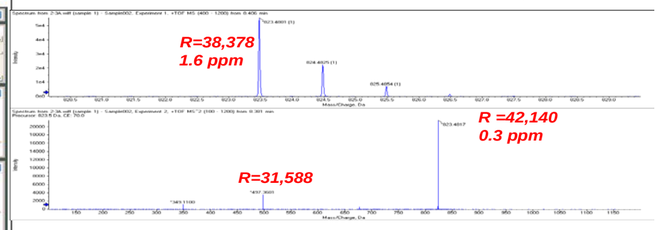
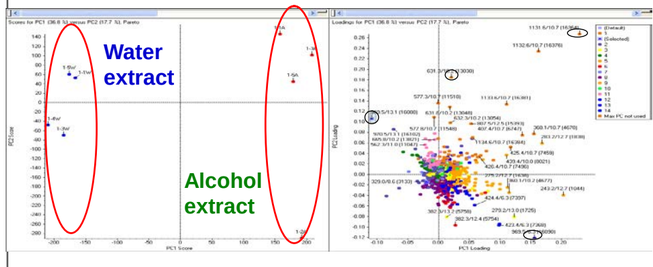


Figure 8: PCA Analysis of Sample Batch 1



A brief study was done to see if water and alcohol extract ginseng differently. Roots were collected from 4 different Ontario farms. PCA clearly shows that these methods generate distinctive profiles. The same approach is being applied to Ginseng samples from various American and Korean regions for genetic and topographic identification.

ACKNOWLEDGEMENT

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TRADEMARKS/LICENSING