TOWARDS QUANTITATIVE SPATIAL PROFILING OF NATURAL PRODUCTS GINSENOSES FROM PANAX GINSENG ROOTS USING MASS SPECTROMETRY IMAGING

Bindesh Shrestha,1 Wei Rao,2 Giorgis Isaac,3 Jimmy Yuk,3 and Mark Ritchie2

1Waters Corporations, Beverly, MA, 2Waters Corporation Shanghai Science & Technology Co Ltd, Shanghai, China, 3Waters Corporations, Milford, MA

ABSTRACT

Panax ginseng C.A. Mey roots have been widely used as traditional herbal medicine in East Asia for several millennia. P. ginseng has been reported to show pharmacological effects on the tumor, immune system, diabetes. Therapeutic utility of ginseng primarily depends on its ginsenosides contents. The effective use of ginsenosides requires profiling the types of ginsenosides and their quantitative distribution in ginseng roots. Mass spectrometry (MS) imaging is gaining momentum for profiling the spatial distribution of natural products in plant tissues. Here, we utilize desorption electrospray ionization (DESI) MS on a quadrupole ToF mass spectrometer (Xevo G2-XS, Waters Corporation, MA, USA) to obtain the spatial distribution of ginsenosides within the ginseng root sections. The frozen transverse cross sections of the ginseng root at 20 μm thickness were prepared for MS imaging. The MS image was plotted and analyzed by High Definition Imaging (HDI) software (Waters Corporation, MA, USA) at 100 μm pixel size. MS imaging was overlaid with microscopic images to define regions of interest (ROI) for three sections in the root cross-section, periderm, cortex, and stele. Fold-change analysis of ion counts in ROI of that section provide quantitative spatial distribution of the ginsenosides. Preliminary analysis shows ginsenosides Rg1/Rh2 were mostly found within the outer bark (periderm) and inner core (stele) of the root. While, ginsenosides Rg1/Rh2, Rg2, Rh1, Rb1, and pseudoginsenoside R1 were more abundant in periderm. Ginsenoside Ra3 exhibited a diffuse distribution within the cross-section and a high concentration around the periderm. To distinguish between isomers, such as Rg1/Rg2, tandem mass spectrometry (MS/MS) with DESI was employed. The characteristic separate fragmentation pattern showing monosaccharide or disaccharide group help discern between the spatial distributions of two ginsenosides. The spatial profile of ginsenosides may differ depending on the age, cultivar, year, geographic origin and quantitative mass spectrometry imaging of ginsenosides from roots can be a helpful tool to study these variations.

INTRODUCTION

- Panax ginseng C.A. Mey roots have been widely used as traditional herbal medicine for several millennia because it contains diverse bioactive natural products, such as ginsenosides, that have been reported to show pharmacological effects on the tumor, immune system, diabetes.
- Since the therapeutic utility of ginseng primarily depends on its ginsenosides contents, it is essential to ascertain the types of ginsenosides and their distribution in ginseng roots.
- Mass spectrometry (MS) imaging is gaining momentum for profiling the spatial distribution of natural products in plant tissues.
- Here, we show the utility of desorption electrospray ionization (DESI) MS on a quadrupole ToF mass spectrometer (to obtain the spatial distribution of ginsenosides within the ginseng root sections).

METHODS

Ginseng Root
Frozen transverse sections of the ginseng, main root of Da-Ma-Ya (a local cultivar of ginseng) was sectioned at 20 μm thickness using a micro-cryotome.

Desorption Electrospray Ionization (DESI) Mass Spectrometry
DESI data were collected on Xevo G2-XS ToF mass spectrometer at negative polarity using MeOH:H2O (9:1) with 0.1 mM NH4Cl and 0.1 mM leucine enkephalin solvent delivered at 1.5 μl/min. DESI imaging at a pixel size of 100 μm with a raster speed of 400 μm/s. The sprayer was supplied with 4.5 V capillary voltage, mass spectrometer used 80 V cone voltage, and mass range m/z 100-2000.

Informatics
Mass spectrometry data were acquired with MassLynx 4.1 and High Definition Imaging (HDI) 1.4 software. All DESI images were rendered and processed by HDI 1.4.

MASS SPECTROMETRY WORKFLOW

UPLC-MS EXTRATION GINSENG ROOT QUADRUPOLE TIME-OF-FLIGHT

Figure 1. Schematic representation of the desorption electrospray ionization DESI-MS imaging on a QToF mass spectrometer. The DESI mass spectrometer is shown. In DESI, nebulized electrospray impinges on tissue surface leading to desorption of ions off its surface in concert with ionization. The ions are detected in a quadrupole mass spectrometer (e.g., Xevo G2-XS). MS imaging is performed by moving the sample stage pixel-by-pixel generating a unique mass spectrum for each pixel.

Figure 2. Ultra-Performance Liquid Chromatography (UPLC) MS workflow for ginsenosides extract analysis compared with DESI imaging workflow for the spatial distribution of the same ginsenosides on the root section using the same mass spectrometer platform, Xevo G2-XS QToF.

Figure 3. Multiplets DESI images of a transverse ginseng root section showing individual ions in the four different color channels are displayed. Without overlaid spatial distribution, it may be hard to discern relative spatial distribution of individual ions, such as m/z 1295 and 533. Individually plotted ions (using heatmap scale) is shown on the right. Scalebar is 3 mm.

Figure 4. The regions of interest analysis (ROI) of periderm (outside), cortex (mid), and stele (inside) portion of the ginseng root can have a different mass spectrum as shown. Demarcation of the ROI is shown on the top right corner. The right side also shows the MS images of abundant ion m/z 377.0845 (maltose) and more localized m/z 987.5521 (pseudoginsenoside R1).

Figure 5. A panel of ions that have higher abundance in the periderm in comparison to the cortex is displayed. Estimated fold-change (labeled as FC) quantitative information was obtained by dividing averaged intensities of two ROIs. Data were normalized to a lock-mass ion with the assumption that lock-mass will be uniform throughout the image.

CONCLUSION

- DESI-MS imaging can be successfully utilized to obtain the spatial distribution of natural products, such as ginsenosides, from sectioned ginseng C.A. Mey roots without much sample prep.
- ROI (regions of interest) analysis with normalization can be applied to obtain quantitative information such as fold-change information between various parts of ginseng root using DESI-MS.

ACKNOWLEDGMENT:
Authors acknowledge the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences in Beijing, China for generously proving the root samples.

©2018 Waters Corporation

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS