TRADITIONAL CHINESE MEDICINE ANALYSIS BY UPLC/ION MOBILITY MASS SPECTROMETRY

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INTRODUCTION

Medicinal herbs, including those used in traditional Chinese medicine (TCM) have been used for many thousands of years. Recently their popularity in Western countries has burgeoned and there has been a shift in the way they are perceived; from alternative therapies to complementary medicines. Hence there is an increasing demand for TCM products to meet/ maintain stringent international quality standards of botanical, chemical and clinical aspects.

The challenges in analyzing Traditional Chinese Medicine (TCM) samples arise from the complexity of the matrix as well as variability from sample to sample. We have reported a generic intelligent work flow[1-2] to allow fast sample analysis while obtaining maximum information by effectively utilizing UPLC/ TOF MS with a variety of novel profiling software tools (MetaboLynx and MarkerLynx). In this poster, we will demonstrate a strategy that provides an effect means of confirming known components and elucidating structures of unknown components.

Ion mobility mass spectrometry (IMS) allows separation of ionic species as they drift through a gas phase under the influence of an electric field. The rate of an ion's drift depends on the mass of the ion, the charge state of the ion as well as the average collision cross-section of the ion. It is possible to separate ions with the same nominal mass if they have different charge states or different collision cross-sections.

The strategy reported here comprises of an initial sample screening using the UPLC/Synapt HDMS in full scan IMS mode. Once a compound or classes of compounds are identified, a targeted fraction collection is performed in analytical scale and the fraction collected can be infused into the mass spectrometer at nano-scale flow rate so that structural elucidation can be performed for the compound of interest. The nano-scale flow rate allows infusion to be carried out over an extended time period, making it possible to conduct multiple MS/MS experiments including the time aligned parallel fragmentation experiments (TAP).

TAP which is CID-IMS-CID allows users to take the advantage of the TriWave configuration on the Synapt HDMS (Figure 1). This configuration allows pre-IMS T-wave and post-IMS T-wave to operate as two separate collision cells. The fragment ions produced in the trap T-wave (pre-IMS) can be separated based on their charge states and sizes as they move through the IMS cell. These ions separated by drift time can be fragmented further in the transfer T-wave (post-IMS). As a result, the fragment ions generated in the transfer T-wave are drift time aligned with their respective precursor ions resulting in Time Aligned Parallel (TAP) fragmentation patterns. When these fragmentation results are combined with software tools such as MassFragment™, structural elucidation for small molecule is simplified.

In this work, a Chinese ginseng extract was analyzed by utilizing the ACQUITY UPLC/Synapt HDMS system operating in IMS mode. The fraction collection was performed using a TriVersa NanoMate from Advion. The fractions collected were directly infused into the Synapt HDMS for analysis providing more in depth information about the compounds of interest. The example analyte discussed in this poster is the Ginsenoside Rb_1.

METHODS

Instrumentation:

LC separation: Waters ACQUITY UPLC® System
MS detection: Waters Synapt HDMS (Figure 1)
Fraction collection and direct infusion:
Advion TriVersa NanoMate with a chip based nano ESI interface.

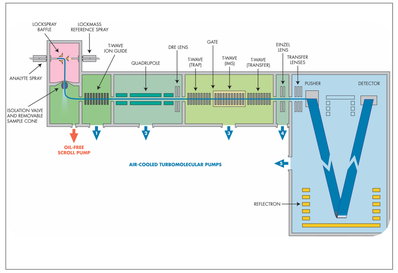


Figure 1. Schematics of the Synapt HDMS instrument configuration.

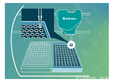
Sample preparation

A Chinese extra strong ginseng drink purchased from JV Trading Ltd, New York was used for this work (Figure 2). The sample was filtered with a 0.45 μm PTFE membrane prior to the LC injection.

Figure 2. The Chinese Extra Strong Ginseng Extract Sample.

Fraction collection (FC) conditions

Flow split: 300 nL/min flow to the MS and the rest of the flow to the waste or to the collection plate when triggered
Collection Plate: 96 well plate
Collection time: 7s per well
Trigger: Time based



METHODS (II)

UPLC conditions:

Column: ACQUITY UPLC HSS T3 Column 2.1 x 100 mm, 1.7 µm, 65 °C
Flow Rate: 600 µL/min.
Mobile Phase A: Water + 0.1% Formic Acid
Mobile Phase B: MeOH
Injection Volume: 5 µL
Strong Wash: AcN/MeOH/IPA/H2O (1:1:1:1)
Weak Wash: Mobile phase A

Gradient:

0 min.	95%A	
10 min.	30%A	Curve 6
17 min.	0%A	Curve 6
20 min.	95%A	Curve 1

MS conditions:

Ionization Mode: ESI Negative
Acquisition Range: 50-1500 m/z
Collision Gas: Argon
IMS Carrier Gas: Helium
He Gas Flow: 80 L/minute

Regular LC/MS interface:			NanoESI interface:		
Capillary V:	3000 V		Capillary V:	0V	
Cone V:	35 V		Cone V:	35 V	
Desolvation T:	450 °C				
Desolvation Gas:	800 L/Hr		Desolvation Gas:	off	
Source Temp:	120 °C		Source T:	60oC	
CE:	Trap:	5 eV	CE for TAP:	Trap:	60 eV
	Transfer:	4 eV		Trans:	70 eV

The fraction collection uses analytical flow at 600 µL/min., even with 300 nL/minute flow goes into the MS, there is still about 599.7 µL/minute flow being used for fraction collection. Each well collects about 70 µL of sample at 7s/well collection setting. The TAP fragmentation for analyte of interest was carried by directly infuse the relevant fraction collected. Each time, 5 µL of sample was used to infuse into the nanoESI chip nozzle at nL/min. scale. The trap and transfer collision energies were raised for data collection (Figure 3).

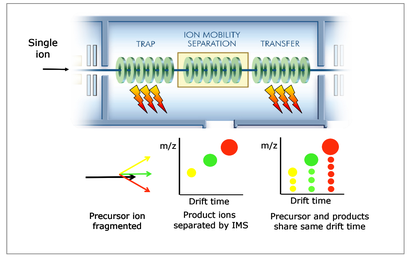


Figure 3. Schematics of the TriWave. Fragment ions can be generated in either/or the Trap and the Transfer region.

RESULTS AND DISCUSSION (I)

Because of the sample complexity, it is desirable to have the compounds of interest physically separated from the raw extract for detailed analyzed. Prep scale chromatography is a common practice for fraction collection for TCM study, however, it is often desirable to determine a component's structure prior to obtain its pure sample physically. In this work, we coupled the UPLC/Synapt HDMS system with an ADVION NanoMate[3-4] to obtain analytical scale fraction collection. Here, the NanoMate was set for the collection of the peak at m/z 1107, which is ginsenoside Rb_1 (structure and major fragments shown in figure 5). As ginsenoside Rb_1 losses the sugar moiety in sequence, it generates fragments of m/z 945, m/z 783, and m/z 621. The m/z 459 is the sapogenin of Rb_1.

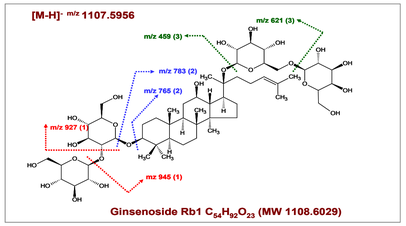


Figure 5 Chemical structural of Ginsenoside Rb_1.

With nL/min flow rate for direct infution, small volume sample can be infused for a long time (ca. 30-40 min.), which allows sufficient time for low level analytes to be analyzed with variety of MS and MS/MS experiments. Figure 6 shows a DriftScope™ comparison from two separate experiments. In 6A, only Trap CE was elevated. Region 1 show mainly $[M-H]^-$ and m/z 945 ion, which is the loss of one sugar ring. Major ions in Region 2 are m/z 945 and m/z 783 (loss of two sugar rings) . And region 3 mainly contains fragment ions generated from the sugars and from the core structural rings.

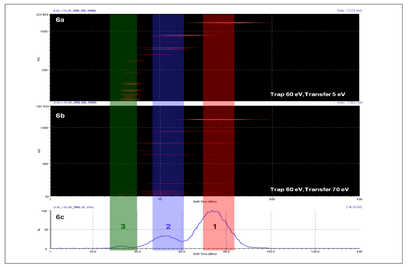


Figure 6. IMS TAP fragmentation results obtained in negative ion electrospray for Ginsenoside Rb1 using different fragmentation strategies.

RESULTS AND DISCUSSION (II)

Figure 6b is the TAP fragmentation result. At each drift time region, the precursor ions were further fragmented producing second generation fragment ions. They are drift time aligned with their precursor ions. This produces a fragmentation tree allowing the user to account for the source of the second generation fragments within the proposed structure. The true advantage of this experiment is that the entire second generation fragment ions can be generated 'on the fly', i.e., in parallel with the generation of the first generation product ions.

The bottom spectrum in Figure 7 shows a combined TAP spectrum of the three regions that correlates to Figure 6b. The top three spectra in Figure 7 show the individual MS spectrum for each region (region 1, 2 and 3) with a few of the proposed structures shown therein. This provides valuable information for the study of the fragmentation mechanisms. For example, It should be noted that the fragment ion at m/z 323, which consists two sugars is observed in drift times 1 and 2, but not 3. This indicates that the precursor ions for region 3 do not have the di-sugar side chain.

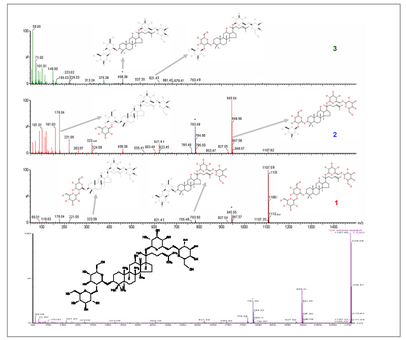


Figure 7. The MS spectrum of Ginsenoside Rb1 by combining the three driftogram peaks from 4c.

CONCLUSION

UPLC/IMS MS with the analytical scale fraction collection combined with TAP fragmentation is complementary to the UPLC/TOF MS workflow previously shown[1-2]. As a result, TCM samples can be analyzed with high resolution, high sensitivity with fast turn around time. This technique enhances the users ability to perform structure elucidation for individual components from complex matrix .
TAP fragmentation in combination with the MassFragment™ structure elucidation tool provides a fast and accurate approach to solving complex elucidation problems.

CONCLUSION

1. K. Yu, J. Castro-Perez, and John Shockcor, Waters Application Note, 2008.
2. K. Yu, Jeremy Nicholson, and John Shockcor, Waters Application Note, 2008.
3. T. Corso, G. Amanda et al., ASMS 2007 Poster.
4. S. Prosser, D. Eliel et al., ASMS 2007 Poster.

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