

## AN INTELLIGENT WORKFLOW FOR TRADITIONAL HERBAL MEDICINE: COMPOUND IDENTIFICATION BY UPLC/TOF MS

Kate Yu, Jose Castro-Perez, and John Shockcor  
Waters Corporation, Milford, MA, U.S.

### INTRODUCTION

Medicinal herbs, including those used in Traditional Herbal Medicine (THM) or 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 THM products to meet and maintain stringent international quality standards of botanical, chemical, and clinical aspects.

Pharmaceutical companies are keen to identify single bioactive compounds that are extracted from THMs. However, the multi-component and synergistic nature of THMs means that it is beneficial to analyze complex extracts. In addition, it is desirable to generate a fingerprint, or a profile, of a given herb/THM to enable differentiation from similar plants, identification of impurities, or a comparison to determine differences occurring due to harvesting times, etc.

THM samples frequently contain hundreds or even thousands of individual chemical entities present in a wide range of concentration levels - and so their analysis is a challenging task. Here we present a generic workflow (Figure 1) using the Waters® ACQUITY UPLC® and SYNAPT™ HDMS™ systems, with its mass spectrometer in time-of-flight (TOF) mode.

UPLC® is a separation technique that offers high resolution, excellent sensitivity, and enables faster analyses. Data generated from MS<sup>E</sup> experiments performed on the SYNAPT HDMS System provides accurate mass information used for predicting the elemental composition of the chemical entities, and fragment ion data for structure confirmation in the absence of pure standards. This UPLC/HDMS system configuration makes analysis of these types of complex samples much easier.

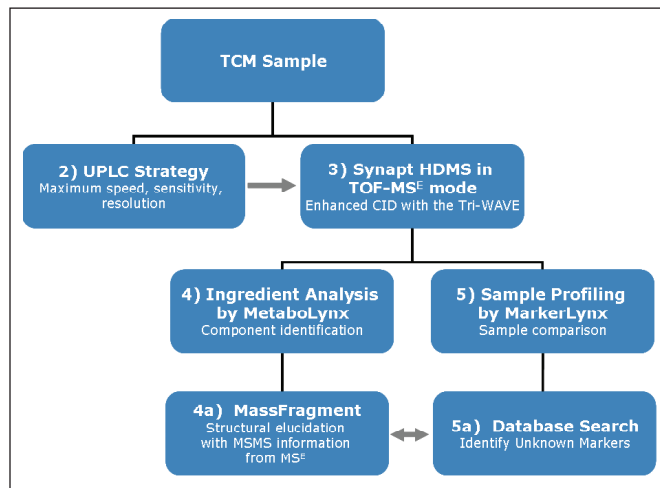


Figure 1. Workflow for THM analysis using UPLC and HDMS (TOF-MS<sup>E</sup>) technologies.

### EXPERIMENTAL

A 5 µL sample of a Chinese Ginseng extract drink was analyzed in the study described. The sample was filtered through a 0.45 µm PTFE membrane and injected without dilution.

#### LC conditions

LC system: Waters ACQUITY UPLC System  
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

| Gradient: | Time   | Composition | Curve   |
|-----------|--------|-------------|---------|
|           | 0 min  | 95% A       |         |
|           | 10 min | 30% A       | Curve 6 |
|           | 17 min | 0% A        | Curve 6 |
|           | 20 min | 95% A       | Curve 1 |

## MS conditions

|                    |                           |
|--------------------|---------------------------|
| MS system:         | Waters SYNAPT HDMS System |
| Ionization mode:   | ESI Negative              |
| Capillary voltage: | 3000 V                    |
| Cone voltage:      | 35 V                      |
| Desolvation temp.: | 450 °C                    |
| Desolvation gas:   | 800 L/Hr                  |
| Source temp.:      | 120 °C                    |
| Acquisition range: | 50 to 1500 m/z            |
| Collision gas:     | Argon                     |

## Data processing

|  |                                 |
|--|---------------------------------|
| Compound screening and identification: | MetaboLynx™ Application Manager |
| Structural elucidation:                | MassFragment™ Software          |

## RESULTS AND DISCUSSION

In a TOF MS<sup>E</sup> experiment, the mass spectrometer performs data acquisition by rapidly switching from a low-collision energy (CE) scan to a high-CE scan during a single LC run.

Figure 2 shows the base-peak ion chromatograms (BPI) obtained from a single ACQUITY UPLC/SYNAPT HDMS TOF-MS<sup>E</sup> experiment. Data was obtained from low-CE scan (bottom), and from the high-CE scan (top).

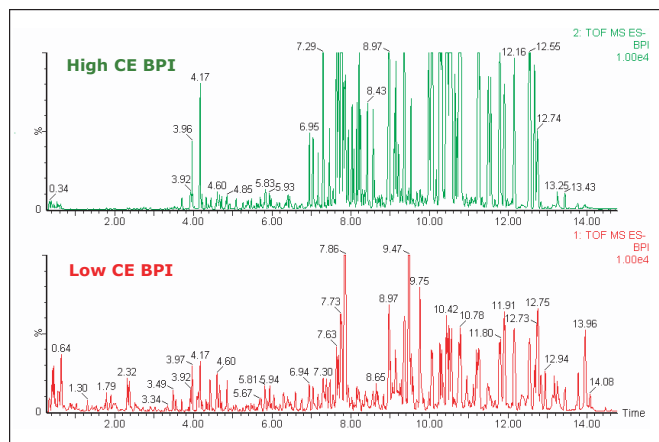
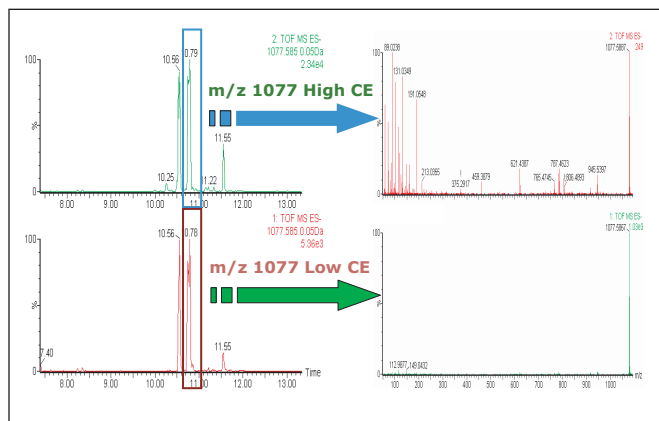


Figure 2. Base-peak ion (BPI) chromatograms of extra-strong Ginseng extract drink from a single UPLC/HDMS TOF-MS<sup>E</sup> analysis, with high- and low-collision energy (CE) scans.

The low-CE experiments provide information about the intact unfragmented ion, e.g. [M+H]<sup>+</sup> (Figure 3, bottom), while the high-CE scan generates fragment ion information (Figure 3, top). Alignment of the low- and high-CE data is automatically performed by the software.



# [APPLICATION NOTE]

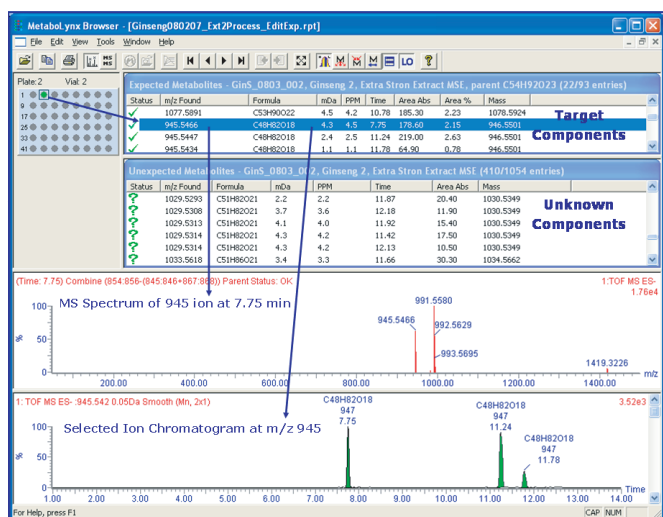


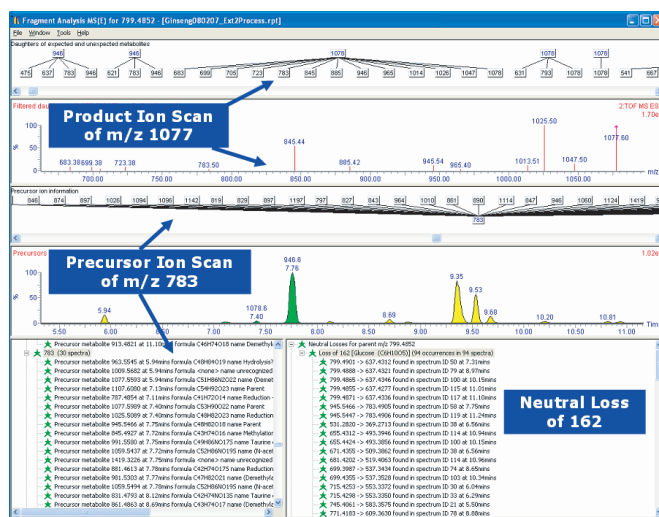
Figure 4. MetaboLynx report for UPLC/HDMS analysis for the Chinese Ginseng extract.

Figure 4 shows a screenshot of the report captured in the MetaboLynx browser, which displays the sample location, and reports the lists of positively-identified expected components as well as detected unexpected components.

More than 400 unexpected components were detected in the analysis of the Ginseng extract drink. The unexpected components are reported with their corresponding retention times, detected m/z values, predicted elemental compositions, exact mass errors, and integrated peak areas.

Structural elucidation of the expected components may be required to confirm its identification. For this, careful examination of the fragment ion information obtained from the high-CE data is necessary.

MetaboLynx aligns the low-collision energy and high-collision energy data such that fragment, precursor-like, and neutral-loss-like ion information can be all extracted from a single injection. The information is displayed in the MassFragment analysis window within the MetaboLynx browser (Figure 5). MassFragment is a chemically-intelligent software tool that facilitates structural elucidations.



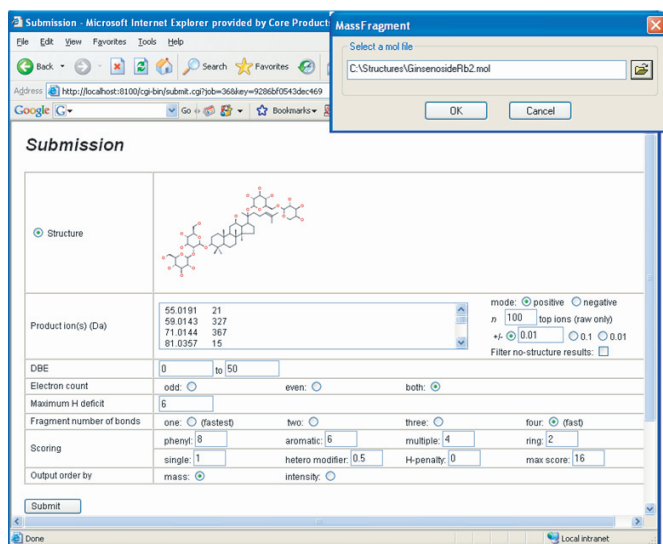


Figure 6. Submission page for structural elucidation by MassFragment.

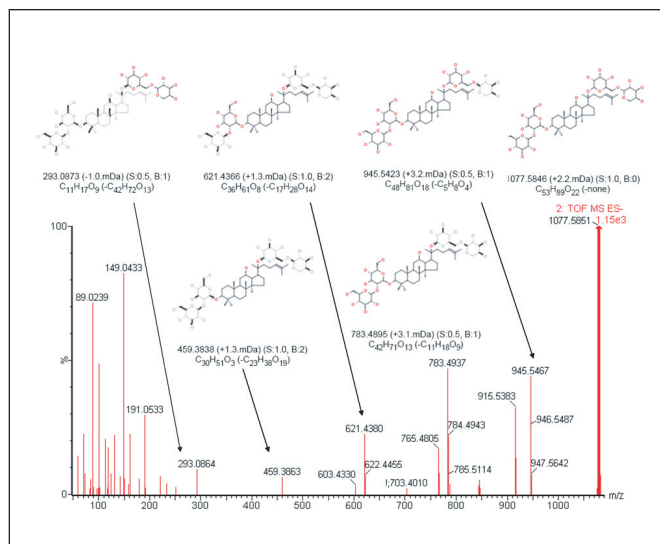


Figure 7. Structural elucidation for ginsenoside Rb2 using MassFragment.

MassFragment assigns a score value for each proposed structure (sometimes more than one structure is proposed). The score is determined by the likelihood of breaking certain types of bonds. Lower scores indicate the most probable systematic bond disconnections based on bond energies. The analyst can choose the correct fragment structures based on score assignment, number of hydrogen added or removed from the structure, as well as the exact mass error.

Once the fragment structures are chosen, a report can be generated and converted into an Adobe Acrobat PDF file. Figure 7 shows an example of the structural elucidation for ginsenoside Rb2 (MW 1078) using the MassFragment tool.



## CONCLUSION

A rapid and powerful approach for Ginseng sample analysis has been described. The ACQUITY UPLC System provides enhanced chromatographic resolution, which enables adequate separation for this very complex sample. Coupling UPLC to the SYNAPT HDMS System allowed for a rapid and accurate sample analysis. The use of MS<sup>E</sup> maximized the information obtained from a single injection and, in the majority of cases, generated confirmation of a putative structure. MassFragment, a chemically intelligent software tool, was key to the efficient structural elucidation process.

This workflow can easily be applied for the analysis of any Traditional Herbal Medicine (THM) or Traditional Chinese Medicine (TCM) sample, enabling them to be analyzed in a much faster time-frame than more traditional protocols, and therefore maximizing overall productivity.

## References

1. Traditional herbal medicine structural elucidation using SYNAPT HDMS with Time-Aligned Parallel Fragmentation. Yu K, Castro-Perez J, Shockcor J. Waters Application Note. 2008; 720002542EN.
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**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)

