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Note d'application

An Intelligent Workflow for Traditional Herbal Medicine: Compound Identification by UPLC-TOF MS

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Abstract

Traditional Herbal Medicine (THM) or Traditional Chinese Medicine (TCM) 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 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^E 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.

Benefits

- A rapid and powerful approach
- Efficient structural elucidation process

- Maximizing overall productivity

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^E 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.

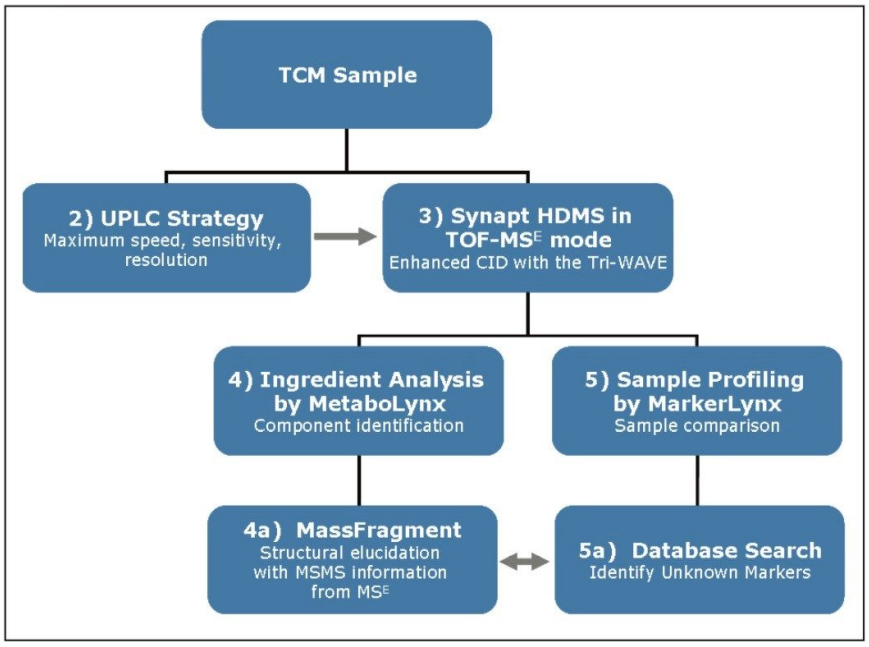

Figure 1. Workflow for THM analysis using UPLC and HDMS (TOF-MS^E) technologies.

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:	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:	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 Management:

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

Results and Discussion

In a TOF MS^E 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^E experiment. Data was obtained from low-CE scan (bottom), and from the high-CE scan (top).

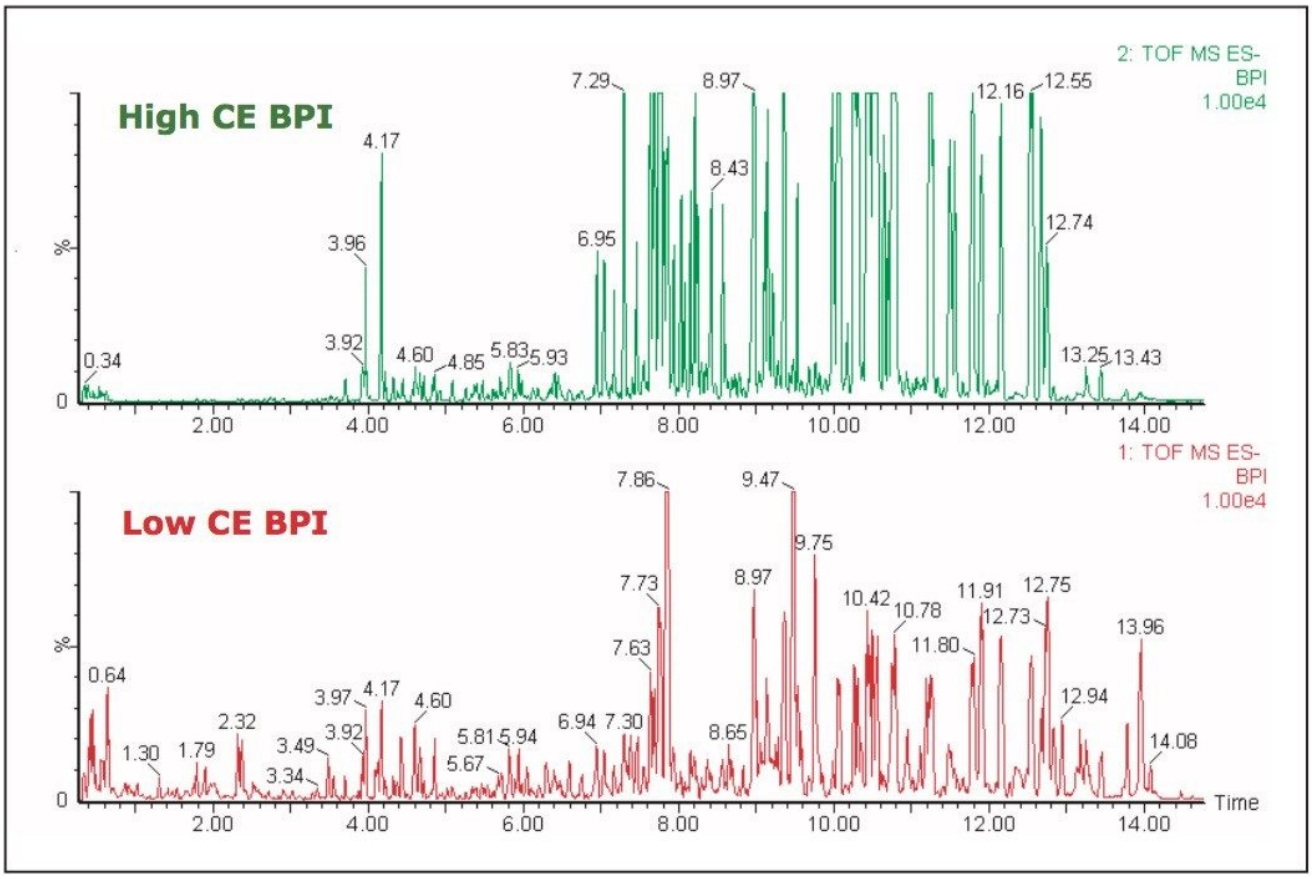

Figure 2. Base-peak ion (BPI) chromatograms of extra-strong Ginseng extract drink from a single UPLC/HDMS TOF-MSE analysis, with high- and low collision energy (CE) scans.

The low-CE experiments provide information about the intact unfragmented ion, e.g. $[M+H]^+$ (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.

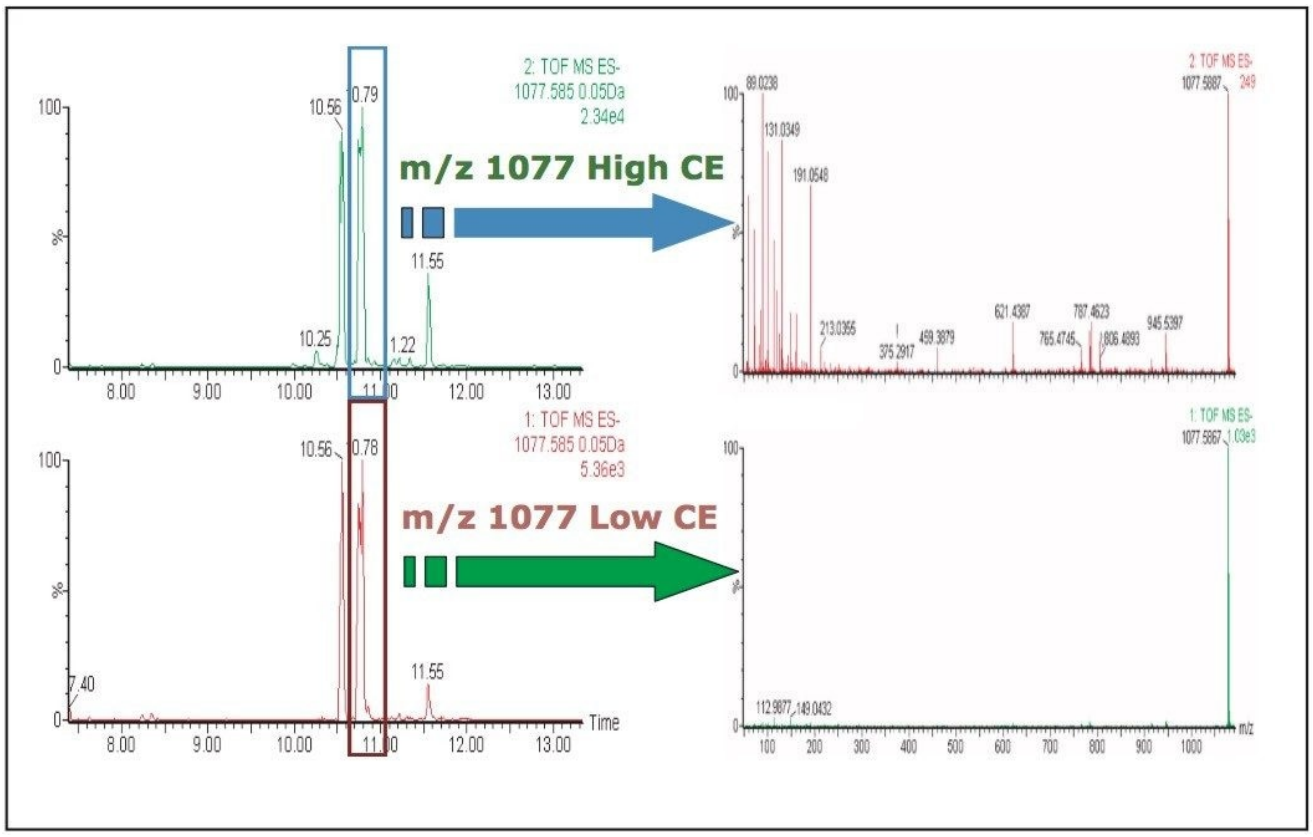

Figure 3. Example result from a UPLC-TOF-MS^E experiment.

The best tool for data mining MS^E results for small molecule compound identification is MetaboLynx, an Application Manager for MassLynx Software. MetaboLynx uses the results from the MS^E low-CE experiments for compound screening (both known and unknown components).[3-4]

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.

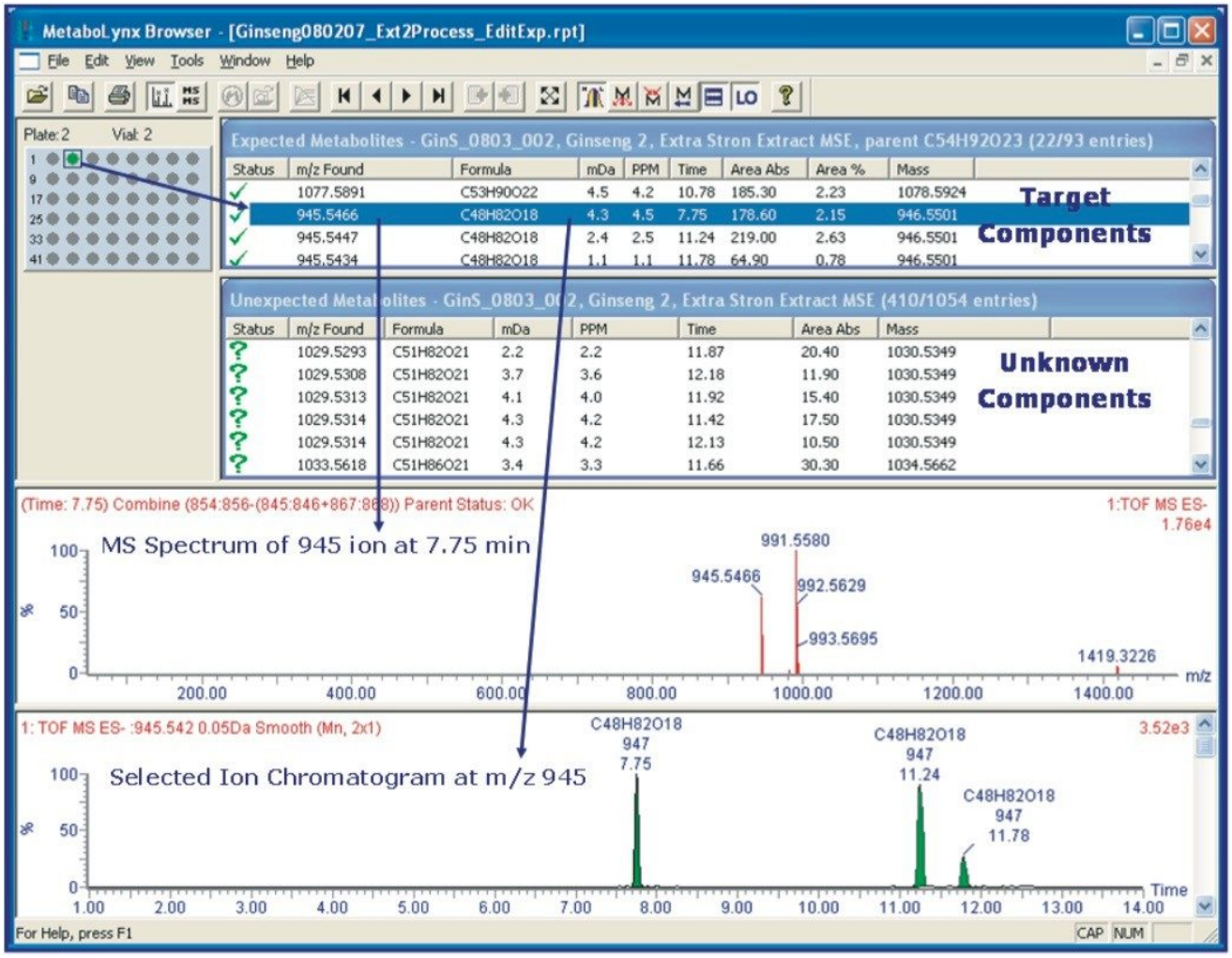

Figure 4. MetaboLynx report for UPLC-HDMS analysis for the Chinese Ginseng extract.

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.

As shown in Figure 5, the upper two windows display the reported fragment ions. The example here shows a result of the fragment ions of a ginsenoside at *m/z* 1077 with *m/z* 783 as one of the fragment ions. The lower part of the window displays precursor ion results as well as the neutral loss information. Figure 5 shows information of the precursor ion of *m/z* 783. One of the precursor ions of *m/z* 783 is *m/z* 1077. Figure 5's bottom right window

also displays information of neutral loss 162. For glycosides, the neutral loss of various sugar rings can be indicative for the structural isomer identifications.

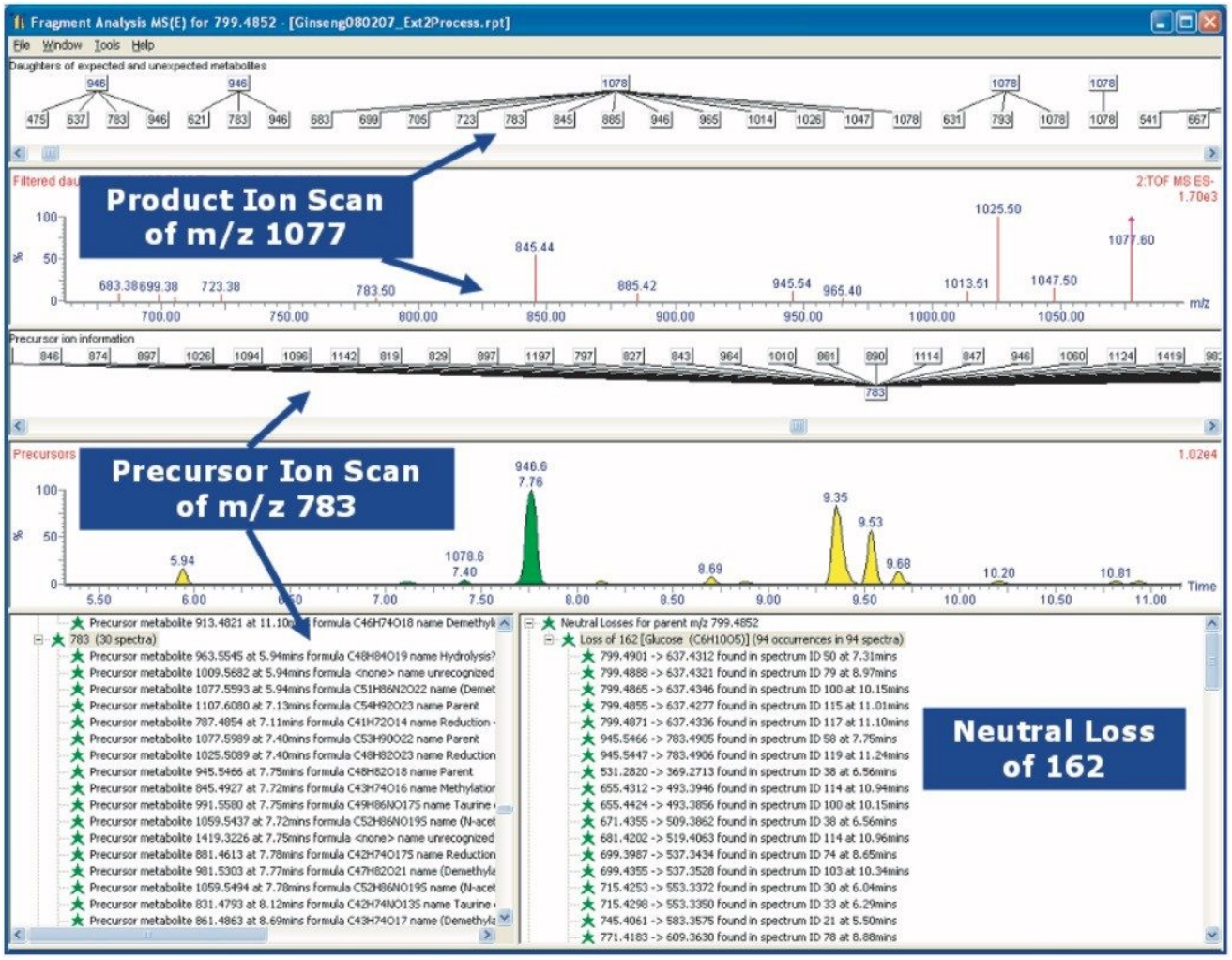

Figure 5. MassLynx fragment analysis window for MS^E data review and data input into MassFragment.

The fragment ion information displayed in the fragment ion scan window can be exported to MassFragment automatically. The proposed structure of the molecule is imported to MassFragment as an MOL file (Figure 6) and is used to assign potential structures for each fragment ion.

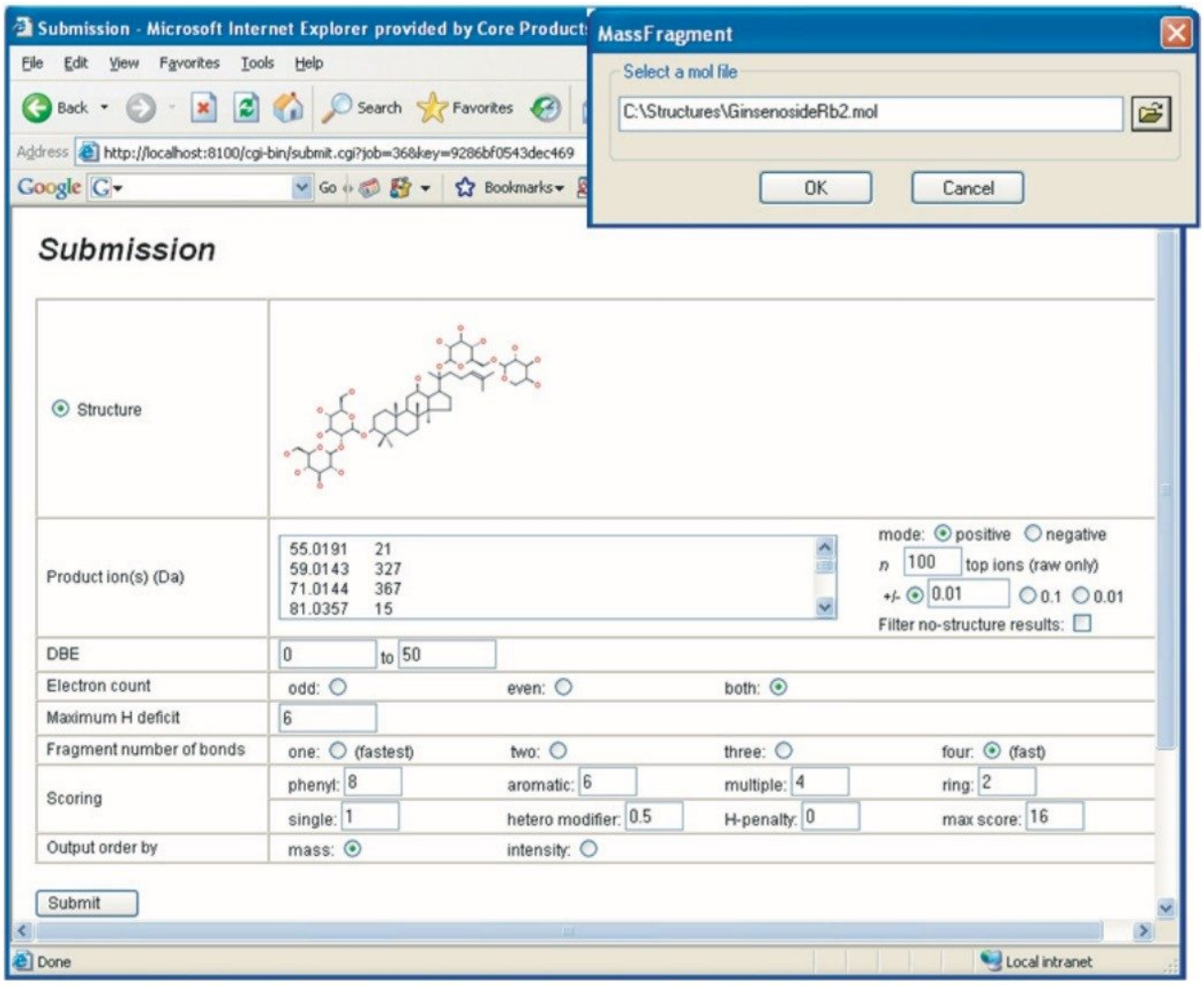

Figure 6. Submission page for structural elucidation by MassFragment.

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. Figure 7. Structural elucidation for ginsenoside Rb2 using MassFragment

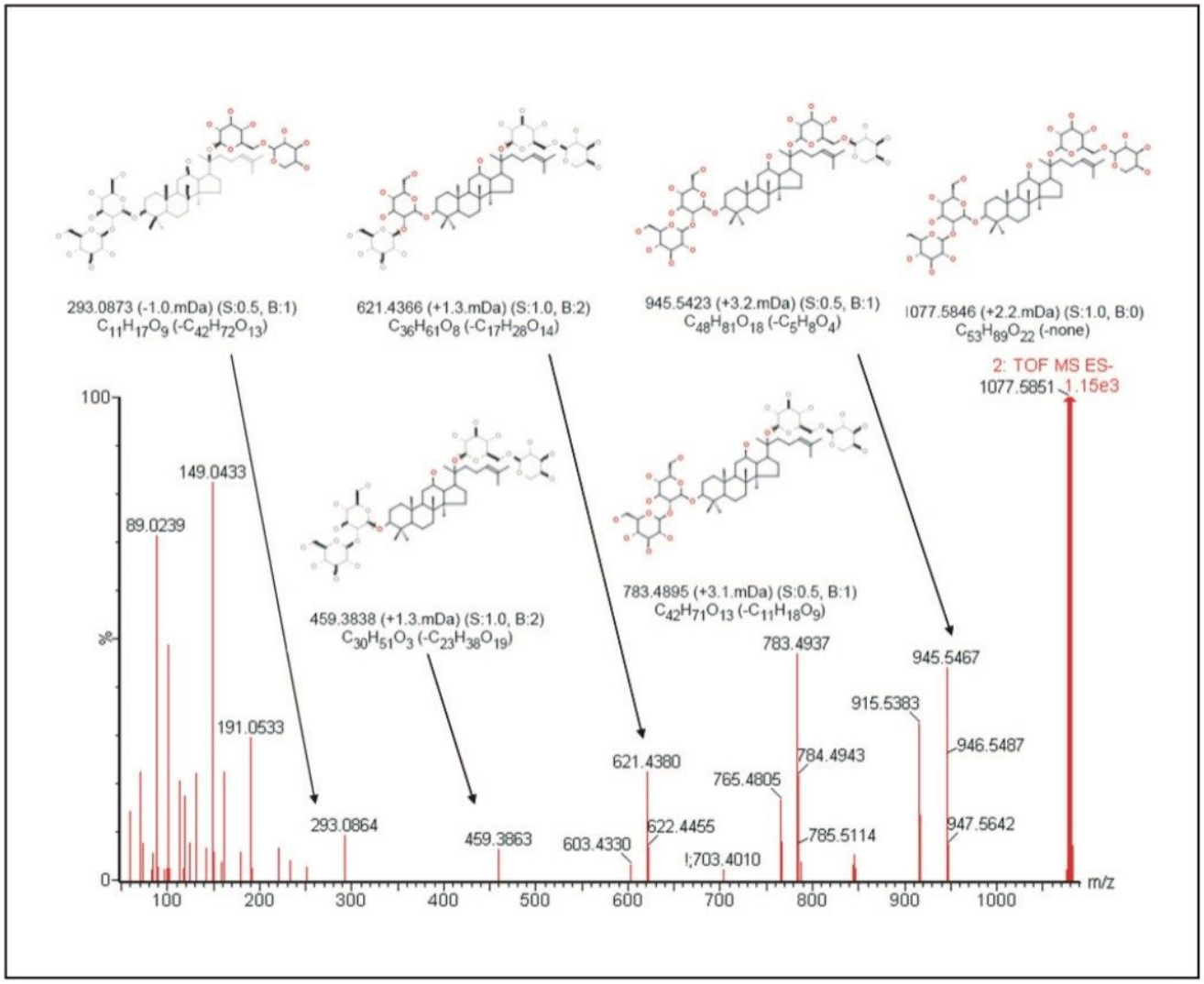

Figure 7. Structural elucidation for ginsenoside Rb2 using MassFragment.

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^E 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 timeframe 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 < https://www.waters.com/webassets/cms/library/docs/720002542en.pdf> .
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