AN INTELLIGENT WORKFLOW FOR ANALYSIS OF NATURAL PRODUCTS: COMPOUND IDENTIFICATION

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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.

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

TCM samples frequently contain hundreds or even thousands of individual chemical entities present in a wide range of concentration levels thus their analysis is a challenging task. Here we present a generic workflow (Fig. 1) using a UPLC-Synapt HDMS system in TOF mode which will increase the ease with which these types of samples can be analysed. UPLC is a separation technique which offers high resolution, excellent sensitivity and enables faster analyses. Data generated from MS^E experiments, performed on the Synapt HDMS, 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.

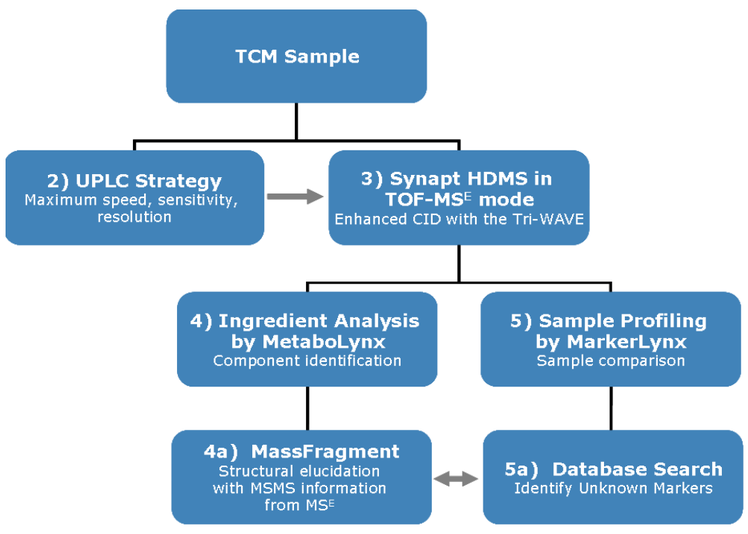

Figure 1. Waters ACQUITY UPLC Synapt™ HDMS TOF MS^E Workflow for TCM analysis.

METHODS

The sample was filtered through a 0.45 µ m PTFE membrane and (5 µL) were injected without dilution onto the column. It was eluted using an optimized gradient of water containing 0.1% formic acid and MeOH. Data were acquired using an external reference to ensure good mass accuracy. Data acquisition was achieved using MS^E (Fig. 2).

LC conditions:

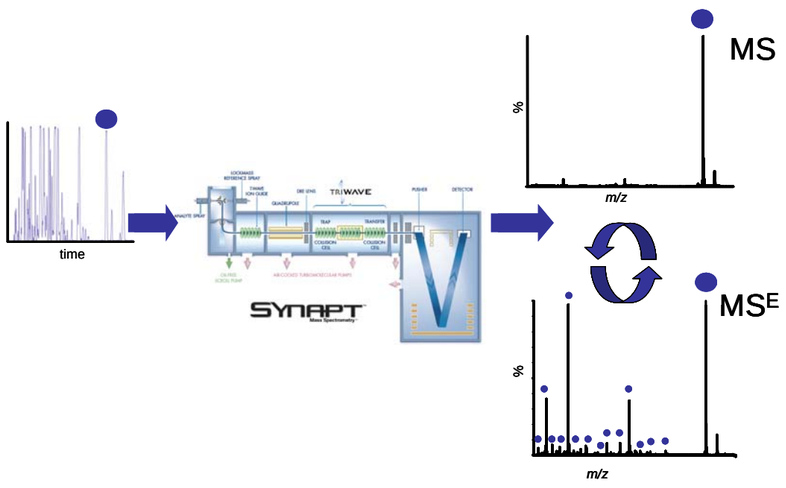

Figure 2. *MS^E acquisition: [M-H]⁻ and fragment ion information by alternating between low and elevated collision energy .*

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

MS conditions

MS System:	Waters Synapt HDMS
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:	50-1500 *m/z*
Collision Gas:	Argon

Data process:

Compound screening and identification:	MetaboLynx™
Structural elucidation:	MassFragment™

RESULTS AND DISCUSSION

Chromatographic resolution and detection of analytes

Data were acquired using a UPLC/SYNAPT HDMS (Fig. 3) in TOF mode using MS^E. The base peak intensity (BPI) chromatograph, (Fig. 4) indicates the presence of numerous analytes, eluted in only 15 min, requiring identification and structural elucidation. Exact mass MS^E data acquisition recorded both low and high collision energy data from the same injection (Fig. 5).

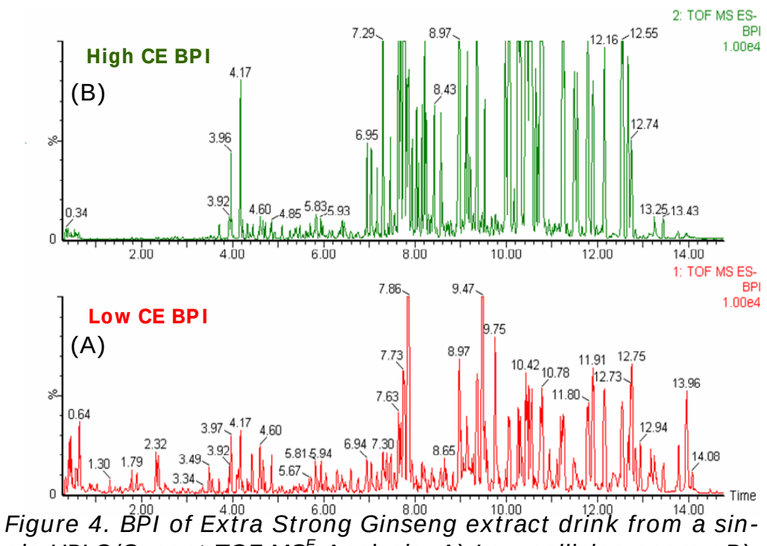

Figure 4. BPI of Extra Strong Ginseng extract drink from a single UPLC/Synapt TOF MS^E Analysis. A) Low collision energy B) High collision energy

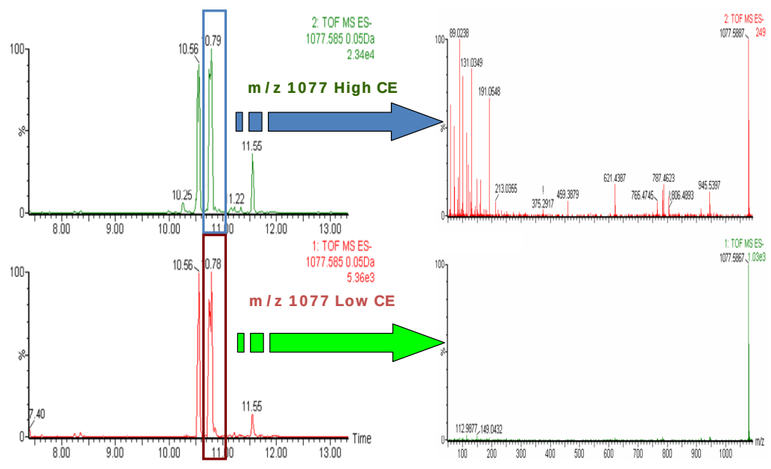

Figure 5. Example result from a UPLC/TOF MS^E experiment.

Identification of analytes using MetaboLynx

MetaboLynx, a MassLynx application manager is used to mine the MS data. MetaboLynx uses the results from the MS^E low CE experiments for compound screening (both known and unknown components).

Fig. 6 shows a screen shot of the report captured in the MetaboLynx ™ browser. In the MetaboLynx™ browser; the sample location, a list of positively identified expected components, and a list of detected unexpected components are reported. 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

MetaboLynx aligns the low and high collision energy data and the fragment data is viewed through the MetaboLynx Mass Fragment Analysis browser. Common fragments and neutral loss information, also displayed in the browser (Fig. 7)

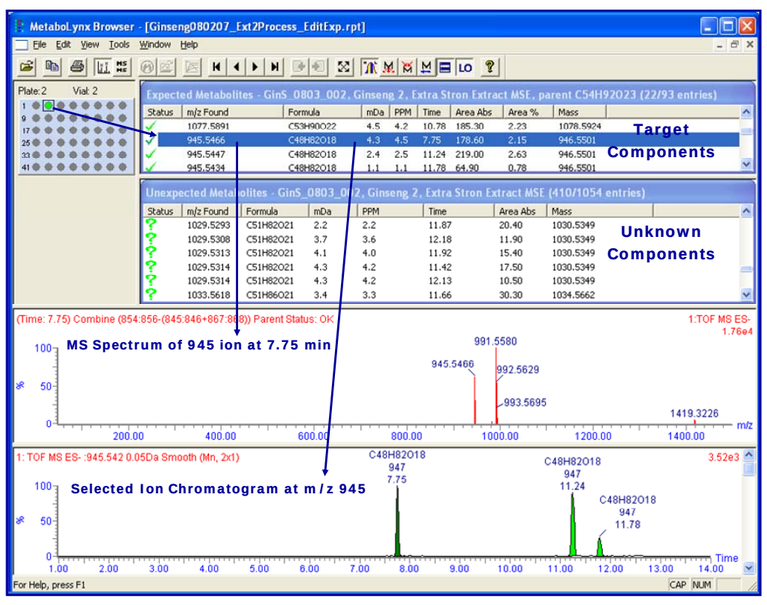

Figure 6. MetaboLynx report for UPLC/Synapt TOF analysis for the Chinese ginseng extract.

Figure 7. MassLynx Fragment Analysis window for MS^E data review and data input into MassFragment

Figure 3. Waters UPLC/Synapt HDMS system .

Assignment of Fragment Ions and Structure Elucidation

The fragment ions in the high energy MS^E spectra can be assigned using MassFragment. This software tool predicts fragment structures based on systematic bond disconnections and a scoring principle. The fragment ion information displayed in the fragment ion scan window is exported to MassFragment™ automatically. The proposed structure of the molecule is imported to MassFragment as a ".MOL" file (shown in Fig. 8) and is used to assign potential structures for each fragment ion. Using MassFragment the key fragments of GinsenosideRb2, were assigned (Fig. 9) within seconds. The assigned fragments consequently aided throughput of complete identification of metabolites.

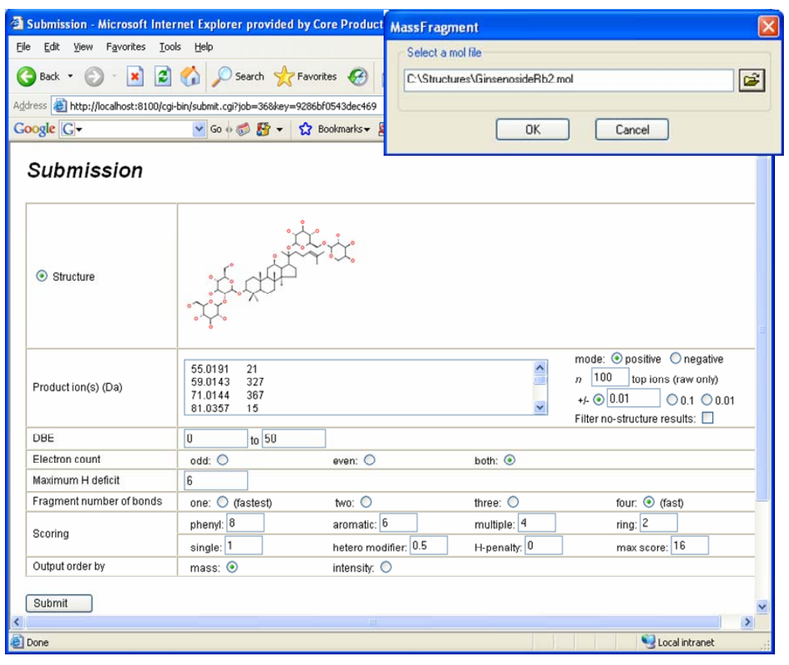

Figure 8. Submission page for structural elucidation by MassFragment

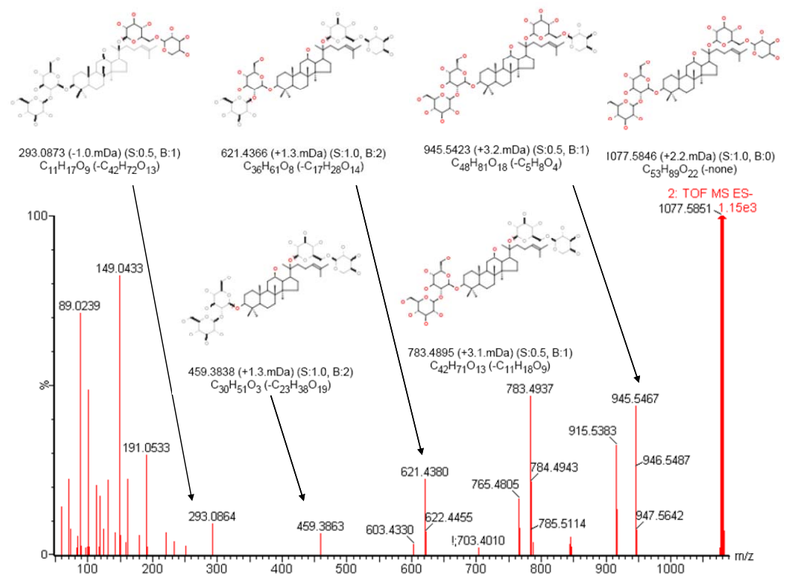

Figure 7. Structural elucidation for ginsenoside Rb2 by MassFragment

CONCLUSION

- **A holistic approach for the analysis of natural product samples is described and applied for the profiling of ginseng**
- **Use of UPLC provided excellent chromatographic resolution in only 15 min, which enabled adequate separation for MS^E analysis of this complex sample.**
- **Coupling UPLC to the Synapt HDMS allowed for a rapid and accurate sample analysis.**
- **Use of MS^E maximized the information obtained from a single injection and in the majority of cases enabled confirmation of a putative structure.**
- **MassFragment, a chemically intelligent software tool, enabled automated assignment of fragment ions from the high energy data. Enabling efficient metabolite structure elucidation**
- **This workflow can easily be applied for the analysis of any TCM samples. Enabling them to be analyzed in a much faster time frame than more traditional protocols. Therefore, maximizing overall productivity.**

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