FAST CHROMATOGRAPHY COUPLED WITH A NOVEL ESI MULTIREFLECTING TOF MASS SPECTROMETER FOR METABOLITE IDENTIFICATION AND NATURAL PRODUCT PROFILING

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INTRODUCTION

Fast chromatographic separations of complex mixtures present a challenging array of near isobaric species. Unlike Fourier-Transform based mass analyzers, the TOF analyzer resolution is independent of data acquisition rate. Elution profiles from fast chromatographic techniques, with peak widths of 2 to 3 seconds at base can be measured without sacrificing mass spectral resolution or losing mass measurement accuracy. To increase the resolution of the TOF analyzer while keeping the overall instrument footprint to a minimum, multipass ion optics using gridless ion mirrors and periodic lenses were used, giving a flight length on the order of 47 m and TOF resolution of greater than 200,000. Since the mass measurement accuracy is dependent on analyzer resolution, sub ppm mass measurement is routine.

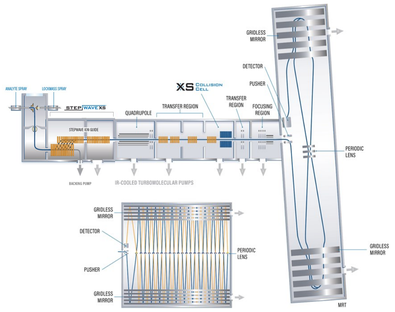


Figure 1. Schematic of the SELECT SERIES™ MRT, a multireflecting TOF with ESI Source

METHODS

A: 0.1% Aqueous Formic Acid

B 0.1% Formic acid in Acetonitrile

Natural Products Profiling CORTECS™ C18 2.1x 100 at 350 µL/min

- **Ramp from 2 to 40 % B in 6 minutes**
- **Ramp to 98% B in 2 minutes**

Metabolite Identification ACQUITY™ HSS T3, 2.1x100 at 350 µL/min

- **Hold 1 minute at 2% B**
- **Ramp to 40 % B in 5 minutes**
- **Ramp to 98% B in 2 minutes**

Mass Spectrometry

ESI + or -, Capillary voltage 2.5 to 3 kV

Desolvation Temperature 450C

Desolvation gas 1000 lph

MRT operated in full flight path mode and tuned to greater than 200,000 res (FWHM) at m/z 556, data collection in MS^E mode using Rapid Encoded Pushing at 5 Hz.

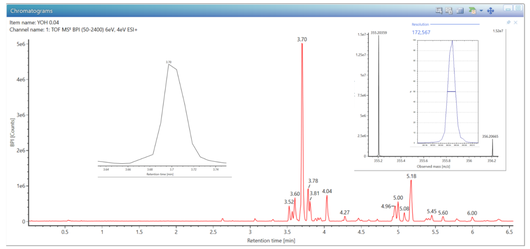


Figure 2. Representative chromatogram of 0.05 mg/mL Yohimbe Bark Extract, 0.5 µL Injection. Left Inset, chromatographic peak for Yohimbine, 2.4 seconds at base. Right Inset Mass Spectra for Yohimbine , resolution is greater than 172,000 (fwhm) at m/z 355.

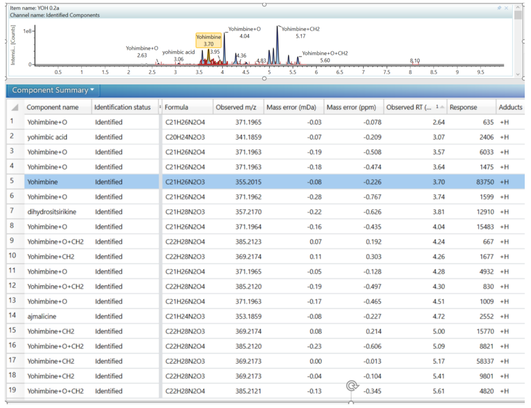


Figure 3. UNIFI™ Summary of Yohimbine and related components[2]. Average mass measurement error for 19 components was -0.263 ppm with an RMS error of 0.394 ppm.

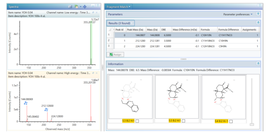
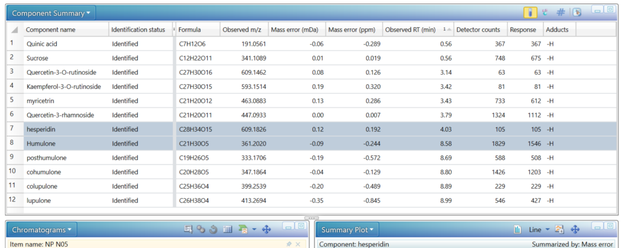


Figure 4. Low and High Energy Spectra for Yohimbe and fragment ion matching using MassFragment. Fragment mass errors were less than 0.1 mDa.

Figure 5. Localization of Yohimbine +Methyl derivative observed at 5.17 minutes.

NATURAL PRODUCTS PROFILING



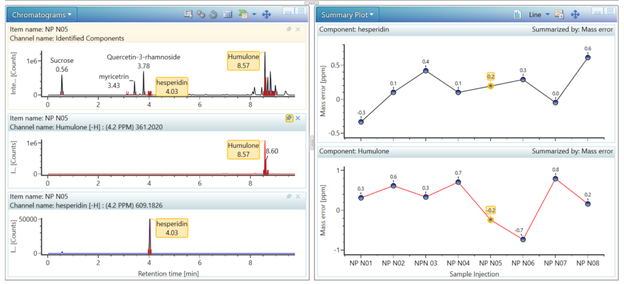


Figure 6. Partial list of Hops alpha acids, flavonoid glycosides, and other components identified in a mixture of Valerian root extract and Hops extract, 0.05 mg/mL, 1.0 µL injection. Average mass error for identified components was -0.134 ppm with an RMS error of 0.374 ppm. The mass error for 8 replicates of Humulone was 0.241 ppm with an RMS error of 0.540 ppm and the mass error for Hesperidin was 0.166 ppm with an RMS error of 0.317 ppm.

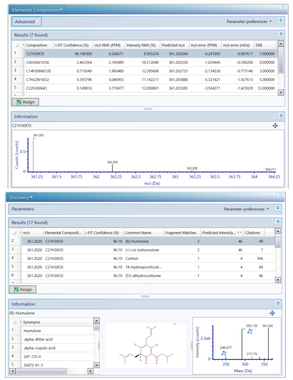


Figure 7. Elemental composition search and ChemSpider Identification of Humulone.

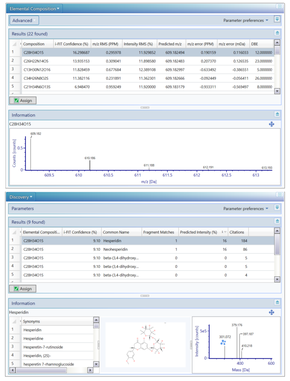


Figure 8. Elemental composition search and ChemSpider Identification of Hesperidin.

METABOLITE IDENTIFICATION

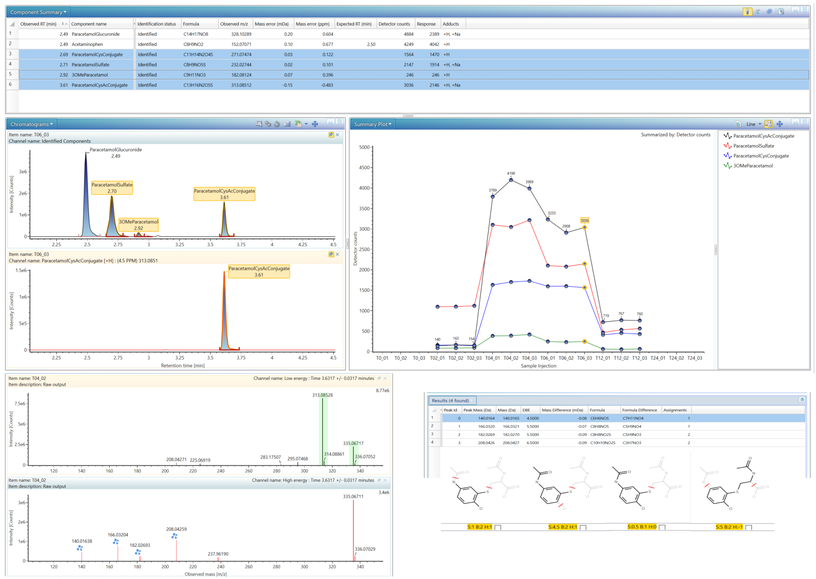


Figure 9. Study of in vivo metabolites of Paracetamol. Metabolites present at 4 hours were identified using accurate mass measurement and fragmentation patterns. The average mass error for the identified metabolites was 0.236 ppm with an RMS error of 0.454 ppm. Timecourses for metabolites are shown, matching established pharmacokinetics for Paracetamol excretion. MS^E Low and High energy spectra for the Acetylcysteine conjugate of paracetamol along with MassFragment report of assigned fragment ions and mass errors.

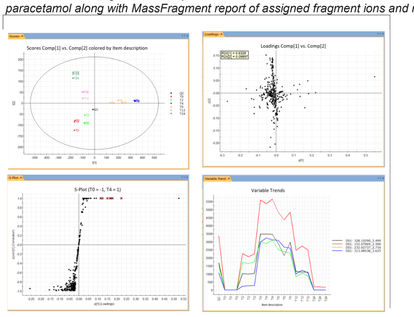


Figure 10. Metabolomic data for in vivo metabolism of Paracetamol, Scores plot Loadings plot, O-PLS S-Plot of ions observed in 4 hour timepoint relative to T0 and selected marker trend plots.

CONCLUSIONS

- **High resolution (>>175,000 fwhm) at greater than 5 Hz data acquisition is ideal for fast chromatographic (2-3 second wide peaks).**
- **Excellent mass measurement accuracy (sub ppm) and repeatability (RMS errors below 1ppm) low for confidence in metabolite identification/'Omics studies and natural product profiling.**
- **High sensitivity and good linearity across more than 3 orders of magnitude.**

References

1 Koslov et al, *Proceedings of the 2017 ASMS Conference*, Indianapolis, IN.

2 Sun et al, *Rapid Comm Mass Spectrom* **2011** 25, 2591-2602.

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