

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.

Item r Chanr	name: YOH 0.2a nel name: Identified Compon	ents		(0				* × ^				
Intensi [Counts]	1e8-	Yohimbine+O 2.63	Yohimi 3.7(3.06	Yohimbine+O 4.04 3.95 4.36	Yohimbine+CH2 5.17 3.Mu Ju Ju 5.	e+O+CH2 60	8 ,10						
<u> </u>	0.5 1	1.5 2 2.5	3 3.5	4 4.5	5 5.5 6 D	6.5 7	7.5 8 8.5	9 9.5	~				
Component Summary *													
	Component name	Identification status	Formula	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (1	Response	Adducts				
1	Yohimbine+O	Identified	C21H26N2O4	371.1965	-0.03	-0.078	2.64	635	+H				
2	yohimbic acid	Identified	C20H24N2O3	341.1859	-0.07	-0.209	3.07	2406	+H				
3	Yohimbine+O	Identified	C21H26N2O4	371.1963	-0.19	-0.508	3.57	6033	+H				
4	Yohimbine+O	Identified	C21H26N2O4	371.1963	-0.18	-0.474	3.64	1475	+H				
5	Yohimbine	Identified	C21H26N2O3	355.2015	-0.08	-0.226	3.70	83750	+H				
6	Yohimbine+O	Identified	C21H26N2O4	371.1962	-0.28	-0.767	3.74	1599	+H				
7	dihydrositsirikine	Identified	C21H28N2O3	357.2170	-0.22	-0.626	3.81	12910	+H				
8	Yohimbine+O	Identified	C21H26N2O4	371.1964	-0.16	-0.435	4.04	15483	+H				
9	Yohimbine+O+CH2	Identified	C22H28N2O4	385.2123	0.07	0.192	4.24	667	+H				
10	Yohimbine+CH2	Identified	C22H28N2O3	369.2174	0.11	0.303	4.26	1677	+H				
11	Yohimbine+O	Identified	C21H26N2O4	371.1965	-0.05	-0.128	4.28	4932	+H				
12	Yohimbine+O+CH2	Identified	C22H28N2O4	385.2120	-0.19	-0.497	4.30	830	+H				
13	Yohimbine+O	Identified	C21H26N2O4	371.1963	-0.17	-0.465	4.51	1009	+H				
14	ajmalicine	Identified	C21H24N2O3	353.1859	-0.08	-0.227	4.72	2552	+H				
15	Yohimbine+CH2	Identified	C22H28N2O3	369.2174	0.08	0.214	5.00	15770	+H				
16	Yohimbine+O+CH2	Identified	C22H28N2O4	385.2120	-0.23	-0.606	5.09	8821	+H				
17	Yohimbine+CH2	Identified	C22H28N2O3	369.2173	0.00	-0.013	5.17	58337	+H				
18	Yohimbine+CH2	Identified	C22H28N2O3	369.2173	-0.04	-0.104	5.41	9801	+H				
19	Yohimbine+O+CH2	Identified	C22H28N2O4	385.2121	-0.13	G ¹ -0.345	5.61	4820	+H				

Figure 3. UNIFI™ Summary of Yohimbine and related components², 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



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NATURAL PRODUCTS PROFILING

Figure 5. Localization of Yohimbine +Methyl

Component Summary 🔹 👘 🐮 # 💽 😐												
	Component name	Identification status	Formula	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min) 1	Detector counts	Response	Adducts		
1	Quinic acid	Identified	C7H12O6	191.0561	-0.06	-0.289	0.56	367	367	-H		
2	Sucrose	Identified	C12H22O11	341.1089	0.01	0.019	0.56	748	675	-H		
3	Quercetin-3-O-rutinoside	Identified	C27H30O16	609.1462	0.08	0.126	3.14	63	63	-H		
4	Kaempferol-3-O-rutinoside	Identified	C27H30O15	593.1514	0.19	0.320	3.42	81	81	-H		
5	myricetrin	Identified	C21H20O12	463.0883	0.13	0.286	3.43	733	612	-H		
6	Quercetin-3-rhamnoside	Identified	C21H20O11	447.0933	0.00	0.007	3.79	1324	1112	-H		
7	hesperidin	Identified	C28H34O15	609.1826	0.12	0.192	4.03	105	105	-H		
8	Humulone	Identified	C21H30O5	361.2020	-0.09	-0.244	8.58	1829	1546	-H		
9	posthumulone	Identified	C19H26O5	333.1706	-0.19	-0.572	8.69	588	508	-H		
10	cohumulone	Identified	C20H28O5	347.1864	-0.04	-0.129	8.80	1426	1203	-H		
11	colupulone	Identified	C25H36O4	399.2539	-0.20	-0.489	8.89	229	229	-H		
12	lupulone	Identified	C26H38O4	413.2694	-0.35	-0.845	8.99	546	427	-H		



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.









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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.