Optimization of Mass Accuracy, Spectral Accuracy, and Resolution for Metabolite Identification Using LTQ-FT Ultra Hybrid Mass Spectrometer in Nano-flow and Normal-flow Conditions

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Introduction

LTQ-FT Ultra hybrid mass spectrometer provides high mass accuracy and high resolution, allowing excellent separation of isotope peaks within isotope clusters. Analysis of isotopic patterns is extremely valuable in elemental composition determination, since previous studies showed that interpretation of isotopic abundance patterns removes more than 95% of false candidate formulas for molecules below 500 Da. It was concluded that instruments with 3 ppm mass accuracy and 2% relative error for isotopic abundance pattern outperformed those with less than 1 ppm accuracy that do not include isotope information in the calculation of molecular formulas. Therefore, optimization of LTQ FT Ultra for the combination of mass accuracy, spectral accuracy, and resolution is critical to metabolite identification. However, few references exist on optimizing high resolution MS to obtain simultaneously the best mass accuracy, accurate isotopic abundance pattern, and spectral accuracy.

Methods

Nano infusion analysis was achieved using a NanoMate nanoESI chip robot (Advion BioSciences, Ithaca, NY) coupled with an LTQ-FT Ultra hybrid linear ion trap – 7.0 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo Fisher, San Jose, CA) operated under Xcalibur software (V2.2, Thermo Fisher). Nanomate holds a 96-well plate, a rack of 384 disposable conductive pipette tips, and a nanoESI chip consisting of a 20 × 20 array of nozzles etched from the planar surface of a silicon wafer. Ten μL of reserpine standard (5 μg/mL) in 0.5% acetic acid in water-methanol (50:50, v/v) were delivered to the back plane of the nanoESI chip with the optimized positive ionization conditions of 1.65 kV and 0.5 psi nitrogen head pressure, so that the reserpine solution in the pipette tip had a constant flow to the chip at a rate of 200 nL/min. For high flow experiments, reserpine standard (1 mg/mL) was infused at 5 μL/min into the ESI source of an LTQ-FT Ultra MS. Survey (full scan m/z 604 – 614) and single ion monitoring (narrow SIM m/z 604 – 614) MS spectra were acquired with the resolution R = 12,500, 25,000, 50,000, 100,000, 200,000, 400,000, 750,000, 1,000,000 (FWHM) at m/z 400 for 1 minute or 20 scans. Mass spectra of the reserpine peak were spectrally corrected using MassWorks (version 2, Cerno Bioscience, Danbury, CT) to achieve high mass and spectral accuracy after data acquisition, using sCLIPS algorithms enabling exact isotope modeling. Elemental composition was obtained using MassWorks, XCalibur embedded elemental composition tool, and Mass Frontier (version 5.1, HighChem Ltd, Bratislava, Slovakia) embedded formula generator tool.

Conclusions

For metabolite identification purpose, LTQ-FT Ultra acquisition in SIM mode with R = 100,000 for 1-minute infusion time may provide optimum mass accuracy, accurate isotopic abundance pattern, spectral accuracy, and resolving power. Because typical HPLC peaks are about 1-minute wide, the same set of parameters may also be applied to HPLC-LTQ-FT Ultra methods. Assuming UPLC peaks are 10-seconds wide, acquisition in SIM mode with R= 50,000 may generate enough data points in typical UPLC-LTQ-FT Ultra runs. Interestingly, full scan acquisition at R = 1,000,000 seems applicable in HPLC-LTQ-FT Ultra conditions. The above conclusions can be applied to either nano-flow LC or normal-flow LC conditions.
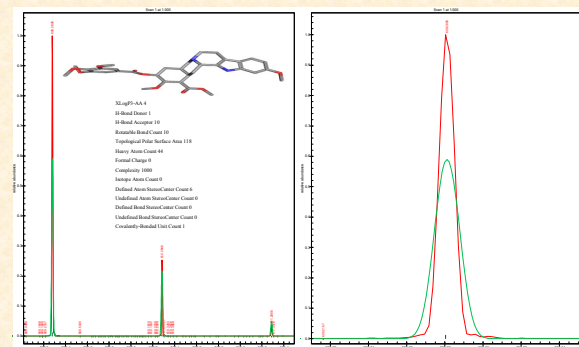
Results

In nano-flow experiments, in order to get spectral accuracy and mass accuracy simultaneously, LTQ-FT Ultra acquisition in narrow select ion monitoring (SIM) mode performed better than in full scan mode with the same scan window (m/z 604-614). In both narrow SIM and full scan modes, as the resolution increased during acquisition, the intensity of average MS spectra decreased, the acquisition time of 20 scans increased, the spectral accuracy decreased, and the rank of the reserpine in the list of all possible candidate formulas also was lowered. But the spectral accuracy decreased faster in full scan mode than in narrow SIM mode. MassWorks always provided the highest rank for reserpine at the low resolution R=12,500 either in narrow SIM or full scan mode. If just for mass accuracy measurement, full scan acquisition plus XCalibur embedded elemental composition tool generated better rank results. In SIM and full scan modes, the mass accuracy, spectral accuracy, and the rank were comparable when using the infusion time of 1-minute instead of 20-scans, at resolutions of 400,000, 750,000 and 1,000,000. Similar results were observed in normal-flow experiments.

Figure 1. Measured isotopic pattern (left panel) and M_0 $[M+H]^+([C_{33}H_{41}O_9N_2]^+)$ (right panel) peaks in red color and theoretical peaks in green color of reserpine standard (5 μg/mL) at R = 100,000 in narrow SIM acquisition mode with scan window of m/z 604-614 for 1-min at 200 nL/min using Nanomate-ESI-LTQ-FT Ultra MS.
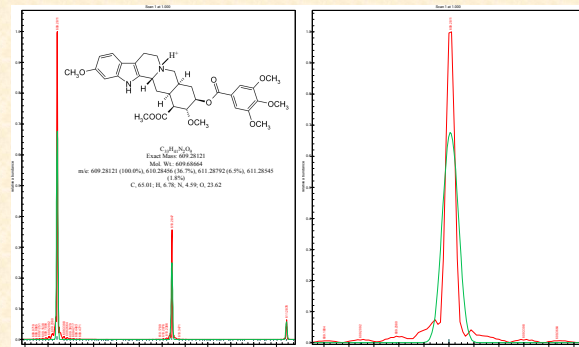
Figure 2. Measured isotopic pattern (left panel) and M_0 $[M+H]^+([C_{33}H_{41}O_9N_2]^+)$ (right panel) peaks in red color and theoretical peaks in green color of reserpine standard (5 μg/mL) at R = 100,000 in full scan acquisition mode with scan window of m/z 604-614 for 1-min at 200 nL/min using Nanomate-ESI-LTQ-FT Ultra MS.

Results (Cont'd)

Table 1. Narrow SIM scans of m/z 604-614 with 1-min duration infusion of reserpine ($C_{33}H_{40}O_9N_2$) standard (5 μg/mL) at 200 nL/min.

Res.	Rank	Software	M0 measured	mDa	PPM	Spectral Accuracy	RMSE	DBE	Scan Time (min)	Average Intensity
12.5k	"1"	MassWorks	609.27834	2.8659	4.7038	97.6467	19,004	14.5	1	9.83 x 10^6
	"14"	XCalibur			-3.80324			14.5		
	"31"	MassFrontier			3.84			14.5-16.5		
25k	"3"	MassWorks	609.27962	1.5859	2.6030	95.9263	23,908	14.5	1	8.72 x 10^6
	"6"	XCalibur			-1.70239			14.5		
	"15"	MassFrontier			1.70			14.5-16.5		
50k	"2"	MassWorks	609.28138	-0.1741	-0.2857	95.5049	12,455	14.5	1	5.86 x 10^6
	"6"	XCalibur			1.18627			14.5		
	"13"	MassFrontier			-1.19			14.5-16.5		
100k	"2"	MassWorks	609.28117	0.0359	0.0590	95.1034	6,105	14.5	1	3.79 x 10^6
	"5"	XCalibur			0.8416			14.5		
	"10"	MassFrontier			-0.84			14.5-16.5		
200k	"4"	MassWorks	609.28130	-0.0941	-0.1544	92.3874	4,270	14.5	1	2.19 x 10^6
	"5"	XCalibur			1.05496			14.5		
	"10"	MassFrontier			-1.05			14.5-16.5		
400k	"4"	MassWorks	609.28168	-0.4741	-0.7781	91.6156	2,028	14.5	1	1.04 x 10^6
	"8"	XCalibur			1.67865			14.5		
	"16"	MassFrontier			-1.68			14.5-16.5		
750k	"4"	MassWorks	609.28162	-0.4141	-0.6796	89.9965	1,331	14.5	1	0.554 x 10^6
	"8"	XCalibur			1.58017			14.5		
	"16"	MassFrontier			-1.58			14.5-16.5		
1000k	"12"	MassWorks	609.28157	-0.3641	-0.5975	81.2153	1,497	14.5	1	0.234 x 10^6
	"6"	XCalibur			1.49811			14.5		
	"12"	MassFrontier			-1.50			14.5-16.5		

Table 2. Narrow SIM scans of m/z 604-614 with 20-scans duration infusion of reserpine ($C_{33}H_{40}O_9N_2$) standard (5 μg/mL) at 200 nL/min.

Res.	Rank	Software	M0 measured	mDa	PPM	Spectral Accuracy	RMSE	DBE	Scan Time (min)	Average Intensity
12.5k	"1"	MassWorks	609.27832	2.8859	4.7366	97.6006	22,335	14.5	0.09	11.3 x 10^6
	"14"	XCalibur			-3.83606			14.5		
	"31"	MassFrontier			3.84			14.5-16.5		
25k	"2"	MassWorks	609.28067	0.5359	0.8796	97.2973	11,780	14.5	0.12	6.72 x 10^6
	"1"	XCalibur			0.02096			14.5		
	"1"	MassFrontier			-0.02			14.5-16.5		
50k	"2"	MassWorks	609.28140	-0.1941	-0.3185	95.3578	14,385	14.5	0.19	6.57 x 10^6
	"6"	XCalibur			1.21909			14.5		
	"12"	MassFrontier			-1.22			14.5-16.5		
100k	"2"	MassWorks	609.28117	0.0359	0.0590	94.9861	7,681	14.5	0.31	4.66 x 10^6
	"5"	XCalibur			0.8416			14.5		
	"10"	MassFrontier			-0.84			14.5-16.5		
200k	"4"	MassWorks	609.28130	-0.0941	-0.1544	92.4307	4,770	14.5	0.57	2.47 x 10^6
	"5"	XCalibur			1.05496			14.5		
	"10"	MassFrontier			-1.05			14.5-16.5		
400k	"4"	MassWorks	609.28168	-0.4741	-0.7781	91.9398	2,238	14.5	1.08	1.19 x 10^6
	"8"	XCalibur			1.67865			14.5		
	"16"	MassFrontier			-1.68			14.5-16.5		
750k	"4"	MassWorks	609.28161	-0.4041	-0.6632	90.0103	1,629	14.5	1.92	0.685 x 10^6
	"8"	XCalibur			1.56376			14.5		
	"16"	MassFrontier			-1.56			14.5-16.5		
1000k	"13"	MassWorks	609.28156	-0.3541	-0.5811	81.1732	1,678	14.5	3.84	0.262 x 10^6
	"6"	XCalibur			1.4817			14.5		
	"12"	MassFrontier			-1.48			14.5-16.5		