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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 isotope patterns is extremely valuable in elemental composition determination; some previous studies showed that interpretation of isotope 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 isotope abundance pattern outperformed those with less than 1 ppm accuracy that do not include isotope information in the calculation of molecular formula. 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 isotope abundance pattern, and spectral accuracy.

Methods
Nano infusion analysis was achieved using a NanoMate nESI 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 Fischer, San Jose, CA) operated under Xcalibur software (V2.2, Thermo Fischer). NanoMate holds a 96-well plate, a rack of 384 disposable pipette tips, and a nanoESI chip consisting of a 20 × 20 array of needles etched from the planar surface of a silicon wafer. Ten µL of reserpine standard (3 µg/mL) was delivered at 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 ES source of an LTQ-FT Ultra MS. Survey scan (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 604 for 1 minute or 20 scans. Mass spectra of the reserpine peaks were spectrally corrected using MassWorks (version 2, Cerno Bioscience, Dunboyne, CT) to achieve high mass and spectral accuracy after data acquisition, using 45LPS algorithms enabling exact isotope matching. 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.

Results
In nano-flow experiments, in order to get spectral accuracy and mass accuracy simultaneously, LTQ-FT Ultra acquisition and narrow scan 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 in either narrow SIM or full scan mode. If not 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.

Figures 1 and 2 show the measured isotope patterns for different resolution, mass accuracy, and spectral accuracy.

Table 1: Narrow SIM scans of m/z 604-614 with 1-minute infusion duration of reserpine (C₃₃H₄₈O₈N₂) 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.

Table 2: Narrow SIM scans of m/z 604-614 with 20-scans duration infusion of reserpine (C₃₃H₄₈O₈N₂) standard (5 mg/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.

Conclusions
For metabolite identification purposes, LTQ-FT Ultra acquisition in SIM mode with R = 100,000 for 1-minute infusion time may provide optimum mass accuracy, accurate isotope abundance pattern, spectral accuracy, and resolving power. Because typical HPLC peaks are about 1-minute width, 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 = 10,000,000 scans applicable in HPLC-LTQ-FT Ultra conditions. The above conclusions can be applied to either nano-flow LC or normal-flow LC conditions.