Combining Profile Mode Mass Spectral Data Processing and Accurate Mass Filtering (AMF) for LC/MS Metabolite Applications

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Introduction

The power of accurate mass (AM) measurement and its application to LC/MS-based metabolite research have been demonstrated by early publications (Ref 1). The data used in these applications are typically not the raw profile mode MS data but rather the processed centroid data, which have many disadvantages due to the heuristic nature of peak picking and mass determination steps employed in the commercial systems. This paper will discuss the many deficiencies in these processing methods and present a novel approach for unbiased and real time profile MS data processing to achieve high fidelity metabolite identification. This new approach will be tested on both a unit mass resolution system and a higher resolution system to demonstrate its utilities and performances.

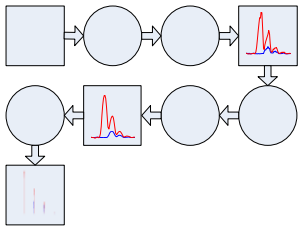
Methods

MS are first calibrated by poly-alanine and/or sodium trifluoroacetate to develop a comprehensive instrument calibration based on Highly Accurate Mass Spectral Calibration Approach (HAMSCA, Ref 2) implemented in MSIntegrity™ software. This calibration is then applied with or without internal standards to each scan in an LC/MS analysis of drugs and their metabolites in the absence and presence of common biological backgrounds. All LC/MS scans are acquired in the profile mode with the lowest possible ion counting cutoff in order to maintain the data integrity. These calibrated LC/MS scans are then subjected to accurate mass filtering to evaluate the capability of both ion retaining and ion rejection. Results from both a unit mass resolution system (4000 QTRAP) and a higher resolution system (QSTAR) are compared through MetIntegrity™ software.

Experiments

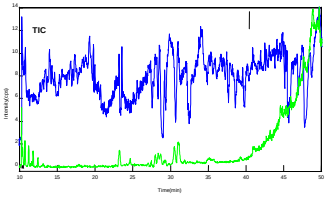
- The metabolites were generated by incubation of rat microsomes with verapamil and separated by C18 column with a gradient and acquired on both AB/Sciex 4000 QTRAP and AB/Sciex QSTAR. The former is a triple stage quadrupole with a linear trap operating in quadrupole mode, and the latter is the hybrid quadrupole and time of flight instrument.
- The incubations were added with rat bile matrix to demonstrate the AM measurements and metabolite profile filtering on samples in the presence of complex background signals.

Calibration Procedures



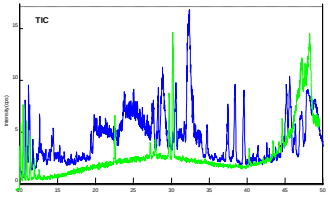
It is first verified that mass spectral scans from an LC/MS experiment are aligned with each other and within a few mDa of true mass values, even on the unit mass resolution 4000 QTRAP instrument. The Extracted Ion Chromatograms (XICs) from both QTRAP and QSTAR are comparable with the correct metabolites identified. The Rejected Ion Chromatograms (RICs) are also comparable on both instruments with the correct matrix ions identified and successfully eliminated. When comparing these results to those with the instrument software processing, it is found that similarly correct XICs and RICs can be obtained from the higher resolution QSTAR only, whereas XICs and RICs from QTRAP are severely contaminated with no clear separation through mass filtering. This indicates that the performance of a unit mass resolution system can be significantly enhanced by the proposed approach (MetIntegrity™), making it possible to perform accurate mass filtering LC/MS experiments on conventional unite LC/MS systems.

TIC of verapamil metabolites with and without Bio-Matrix on 4000 QTRAP

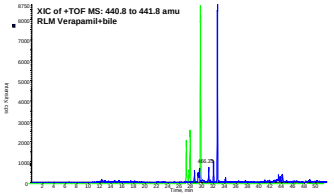


Results and Discussion

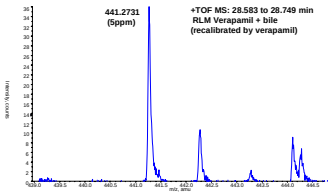
TIC of verapamil metabolites with and without bile matrix on QSTAR



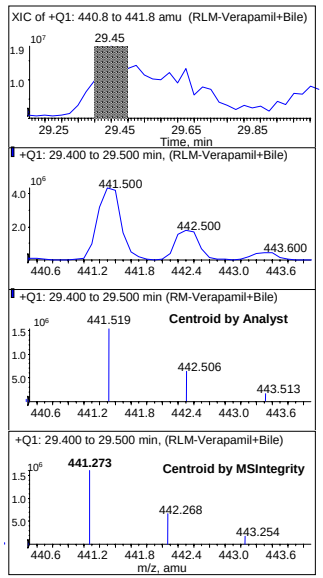
XIC of verapamil metabolites with and without bile matrix on QSTAR

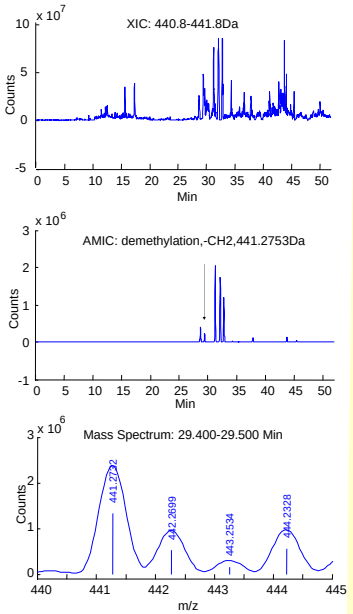


AM of demethylated verapamil with bile matrix on QSTAR



Conventional XIC and Filtered XIC with Accurate Mass Identification on 4000 QTRAP





AM of verapamil & demethylated verapamil

Name	Ion Formula	Calc. Mass
Verapamil	C27H39N2O4	455.2910
Demethylated Verapamil	C26H37N2O4	441.2753

Conclusions

- Proper mass spectral processing in profile mode is critical to mass accuracy.
- With profile mode MS processing and AMF, unit mass resolution LC/MS can achieve effective ion extraction and ion rejection in routine metabolite studies with similar performance as obtained on a higher resolution qTOF system, even in the presence of complex bio-matrices (bile).
- LC/MS-based metabolite identification can benefit from mass spectral processing and accurate mass filtering.

References

1. Haiying Zhang, *J. Mass Spectrom.* 2003; **38**:1110-1112.
2. Ming Gu, Yongdong Wang, and Don Kuehl, *Spectroscopy* (*Current Trends in Mass Spectrometry*), 2005; 22-26

Run External Calibration Standard

Statistically examine peak shapes and mass errors