Comparing Mass Defect Filtering and Accurate Mass Profile Extracted Ion Chromatogram (AMPXIC) for Metabolism Studies

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Introduction

In LC/MS experiments, Total Ion Chromatogram (TIC) provides a good summary of ions being separated/detected. While usually very informative, TIC works best when the analytes are dominating the ion signals in any given retention time window. In the presence of many other ions of similar abundance levels, however, TIC may become too complex to be informative, giving rise to eXtracted Ion Chromatogram (XIC), which only integrates ion intensities falling within a given mass window (±0.5Da). For more complex samples such as those collected with bile matrix, more selective filter is needed for effective metabolite identification. Three such filters will be studies and compared in this paper: Accurate Mass eXtracted Ion Chromatogram (AMXIC), Mass Defect Filtering (MDF, Ref 1) and Accurate Mass Profile eXtracted Ion Chromatogram (AMPXIC™, Ref 2), all using MS data at unit mass resolution with the high mass accuracy calibration available through MassWorks calibration.

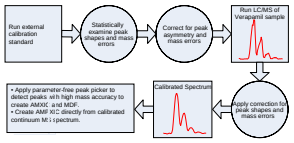
Methods

For biofluid samples with many background ions, the ions of interest will typically be obscured in the TIC. Even when processed into XIC, some of these background ions and their isotopes would happen to have their nominal masses fall within the XIC integration window of ±0.5Da, resulting in a rather complex XIC. With the high mass accuracy capability of MassWorks™ software, a more selective Accurate Mass XIC (AMXIC™) can be generated in a tight accurate mass window of ±15mDa even on a unit mass resolution MS system. AMXIC, however, does not take into consideration of the isotope clusters and their isotope profiles, resulting in a sub-optimal tradeoff between sentitivity and selectivity. A novel filter based on both accurate mass and isotope profile, AMPXIC, is implemented and tested along with Mass Defect Filtering (MDF) on Verapamil incubation samples with varying degrees of background ions from clean incubation to bile matrix.

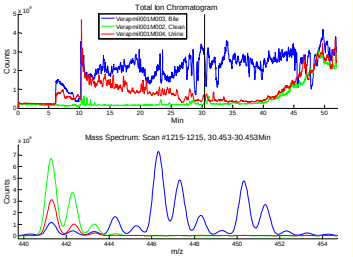
Experiments and Data Processing

- The metabolites were generated by incubation of rat microsomes with verapamil and separated by C18 column with a gradient and acquired on AB/Sciex 4000 QTRAP instrument, a triple stage quadrupole with the 2nd stage quadrupole operating as a linear ion trap.
- The incubations were analyzed with LC/MS as is, after addition of rat bile or urine matrix, resulting in a total of three LC/MS runs to demonstrate and compare the various approaches for processing ion chromatograms with varying background levels.
- Prior to the LC/MS analysis, a standard mixture containing 8 different drugs covering 100-800Da mass range is injected through a loop with a mobile phase and flow rate similar to those for the LC/MS analysis.
- The MS scans acquired from the loop injection are used for a comprehensive mass spectral calibration involving both the mass axis and MS peak shape using the MassWorks software.
- Each MS scan in an LC/MS run would then be calibrated using this comprehensive MS calibration prior to ion extraction analysis via AMIC, MDF, and AMPXIC.

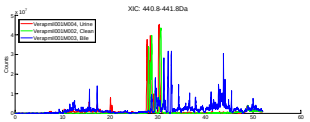
MassWorks Calibration & Data Processing



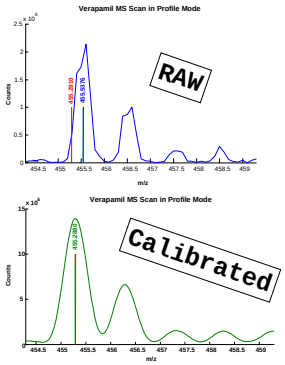
Results and Discussion



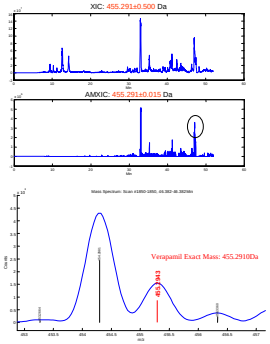
For clean incubation, TIC shows clearly the ions of interest as chromatographic peaks. For a more complex with urine background, its TIC becomes too complex to be useful. For the most complex bile sample, all ions of interest becomes invisible in its TIC, in spite of the high concentrations used.



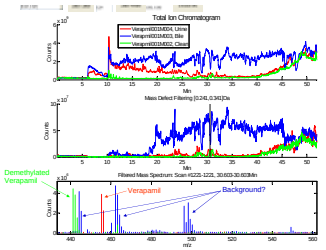
While still useful in revealing ions of interest in the presence of moderately complex urine sample, XIC now contains false positives already. For the most bile sample, the contamination becomes so severe that it is hard to discern the ions of interest amongst all the false positives, especially at lower concentration levels.



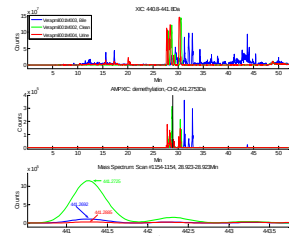
With the high mass accuracy available after calibration, it is now possible to perform AMXIC on unit mass resolution data in an attempt to reduce or eliminate the contribution of matrix ions to the extracted ion chromatogram.



It is seen that AMXIC can indeed simplify the extracted ion chromatogram due to the more accurate mass window used. When applied to the most complex bile sample, it is also clear that other matrix ions still make into the extracted ion chromatograms, including the example shown above when the M+1 isotope of a matrix ion makes into the AMXIC as the determined accurate mass for M+1 cluster happens to fall within the accurate mass window.



Due to the high mass accuracy available through MassWorks calibration, MDF, developed specifically for high resolution systems, can now be applied to the three LC/MS runs with unit mass resolution in a ±50mDa mass defect window. For the clean incubation, very little difference is seen between TIC and MDF Ion Chromatogram (MDFIC) in terms of peak patterns, though the overall background level is reduced by ~10x. Towards the end of the LC run where most of the matrix ions appear, significant portions of these matrix ions have their mass defects within the given window, resulting in a rise in baseline much like that in the TIC. The benefits of MDF is most clearly seen in the urine sample where significant improvement in peak patterns is achieved through filtering of the background ions prior to RT=30min, resulting in MDFIC very similar to that from the clean sample. When applied to the bile sample, however, MDF does not yield much more information than the TIC, due possibly to the level and variety of the matrix ions involved, as evidenced in the filtered mass spectrum at the bottom.



Different from MDFIC, AMPXIC requires a list of all possible biotransformations which can then be searched throughout the whole calibrated LC/MS data set to create a filtered ion chromatogram matching the exact mass and isotope distribution of the postulated ion. As seen from the above graph for the example of demethylation metabolite, XIC from the clean sample offers a clear picture of the few demethylation metabolites while XICs from the urine and bile samples are clearly contaminated. With mass accurately calibrated and MS peak shape mathematically matched, AMPXICs for all three samples, including the most challenging bile sample, show the same clear chromatographic peak patterns, subject only to retention time shifts amongst the three LC/MS runs, demonstrating highly selective filtering power of AMPXIC processing. Furthermore, the corresponding mass spectrum after the calibration can be reliably analyzed for the purpose of peak detection and peak integration, resulting in accurate masses and unbiased peak area integration for confirmation and quantitative analysis.

Conclusions

- TIC is only useful in revealing ions of interest when there is no significant background.
- XIC suffers from both chemical noise as well as isotope interferences and could not reveal ions of interest in complex matrices.
- AMXIC is significantly more selective than XIC but still suffers from significant false positives in complex matrices, especially towards the end of LC/MS run.
- MDF is useful for clean and moderately complex samples but becomes as featureless as TIC for a truly complex sample.
- AMPXIC is able to handle various types of matrices but does require a list of possible bio-transformations *a priori*.

Acknowledgement

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References

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