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MsXelerator RM: A Software Platform for Reactive Metabolite Detection using Low and High Resolution Mass Spectrometry Data

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INTRODUCTION

As a leading cause of patient morbidity and mortality, idiosyncratic drug reactions (IDRs) remain a major and increasing safety concern both in clinical drug development and after market launch of the drug. To date it is especially difficult to predict IDRs, because they have a very low frequency of occurrence, no obvious dose-response relationship, and no universal animal model for evaluation. Although mechanisms of drug-induced idiosyncratic hepato-toxicity remain to be elucidated, there is a substantial amount of evidence that implicates chemically reactive metabolites as toxicity mediators.

Current analytical strategies to detect and identify reactive metabolites are all based on indirect measurements by LC/MS, largely because reactive metabolites are electrophiles that can be captured by a **trapping** molecule such as glutathione (GSH) to form stable adducts in microsomal incubations.

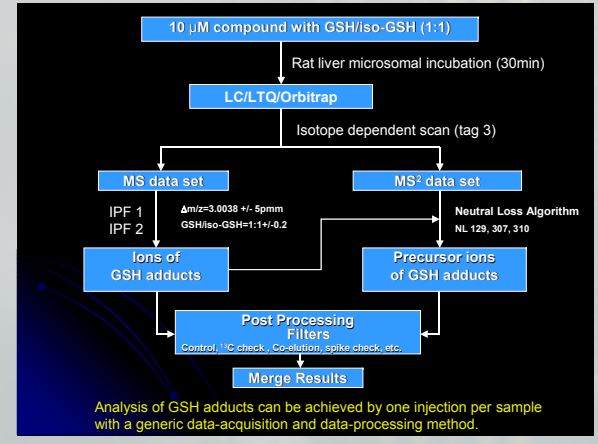
Recent approaches for the detection of GSH adducts using high resolution mass spectrometry include:

- Mass Defect Filtering (MDF)
- High Resolution Neutral Loss Filter
- High Resolution Background Subtraction
- Isotope Pattern Searching with MDF

Recently we reported a new approach and algorithms for screening GSH adducts using the isotope trapping technique, in which a mixture (1:1) of natural and stable-isotope-labeled glutathione (GSH-$^{13}C_2$-^{15}N) is used to trap reactive metabolites. The GSH adducts formed are detected by the presence of a unique isotopic doublet (mass difference of 3.0037 Da, ratio 1:1) using the **Isotope Pattern Filtering Algorithm** (IPF) from MsXelerator™.

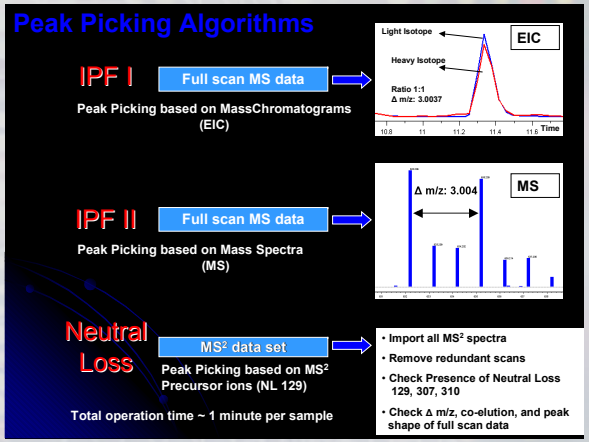
Here, we present a new Software Platform, **MsXelerator RM**, that combines **IPF, Neutral Loss Filter** and **Background Correction** into one method. It was developed to automate the above techniques and to decrease the chance of missing important reactive metabolites by combining different data analysis methods. Special post-processing filters are included that will automatically filter false positives from the list of initial hits.

FLOW CHART OF ANALYSIS OF GSH ADDUCTS

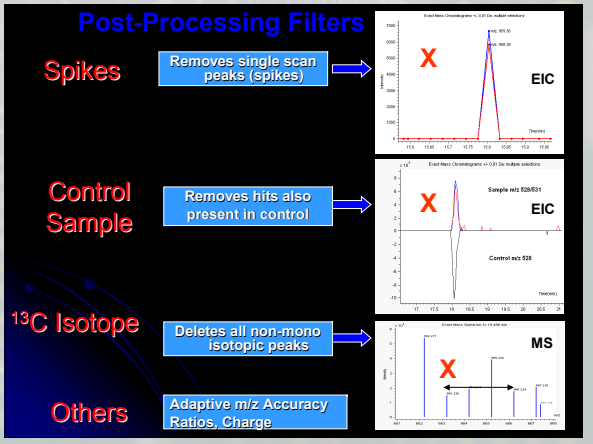


* Different natural/labeled trapping reagents can be used, e.g. GSH, KCN, MOA
** IPF I & II can be combined with Mass Defect Filtering (MDF)

IPF AND NEUTRAL LOSS WORKFLOWS

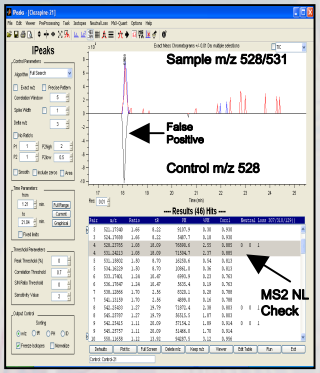


FALSE POSITIVES : POST-PROCESSING FILTERS

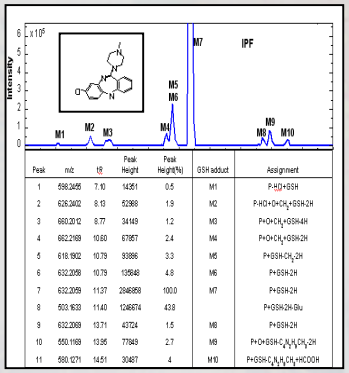


Results from IPF and Neutral Loss Filtering need to be checked using a number of additional filters to remove possible false positives. This is especially true if peak picking was performed using no intensity thresholds (high sensitivity) or when using the IPF II algorithm – Direct Raw MS data search

GSH ADDUCT SCREENING – RESULTS OF IPF AND NLF



Result screen for GSH adducts of Clozapine. Initial run detects 46 hits. After applying the post-processing filters only 11 GSH adducts remain. Shown is one of the false positives. The false positive also shows a Neutral Loss of 129 !!

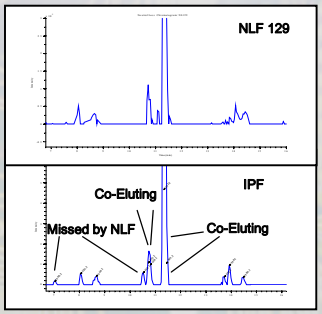


One single IPF run can achieve identification of a variety of GSH adducts. Shown are the high resolution reconstructed IPF TIC and the results for Clozapine

Summary of GSH Data Mining on 4 test compounds

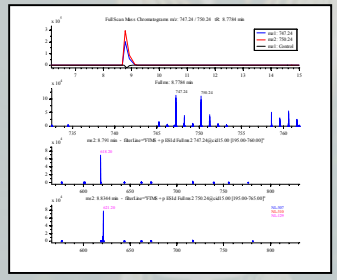
	clozapine	diclofenac	imipramine	ticlopidine
EIC (extracted Ion chromatogram)	3	3	7	12
MDF (Mass defect filter)	5	6	6	19
NLF (Neutral loss filter)	4	5	4	12
PIF (Precursor ion filter)	1	1	1	5
IPF (isotope pattern filter)	10	7	10	19

→ Combine results of IPF and NLF when different



Top: Neutral Loss 129 Full Scan Reconstruction
Bottom: IPF Reconstruction – IPF shows much more selectivity. Peaks are automatically identified and linked with the results table for viewing of mass spectra and mass chromatograms.

Reporting: GSH Overview Plot



→ EIC: overlay of light and heavy EIC pairs, combined with light EIC of control sample. Full scan or MS2 scan

→ Mass Spectra at retention time, showing accurate m/z difference of 3.0038

→ MS2 spectrum of light isotope > Neutral Loss identified and marked

→ MS2 spectrum of heavy isotope > Neutral loss identified and marked (shows 3 Da difference compared to MS2 spectrum of light isotope, due to loss of m/z 129

The MsXelerator RM platform enables high resolution LC/MS/MS to perform higher throughput screening of reactive metabolites in a drug discovery setting. The IPF approach is capable of sensitive and comprehensive detection and characterization of GSH adducts. Post-processing filters are definitely needed to remove false positives. NLF offers an additional information source and is need for low resolution instruments.