IPeaks: Isotope Pattern Matching for Fast and Sensitive Drug Metabolite Detection using High Resolution Mass Spectrometry

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MsMetrix
Accelerating Data Analysis in LC/MS

INTRODUCTION

High Resolution Mass Spectrometry combined with labeling strategies is a powerful method to discriminate real metabolites containing a specific isotopic pattern from all other matrix compounds in a sample. Current Isotope Filtering techniques mostly remove non-matching ions from the data set. It still takes quite some time to explore the remaining data.

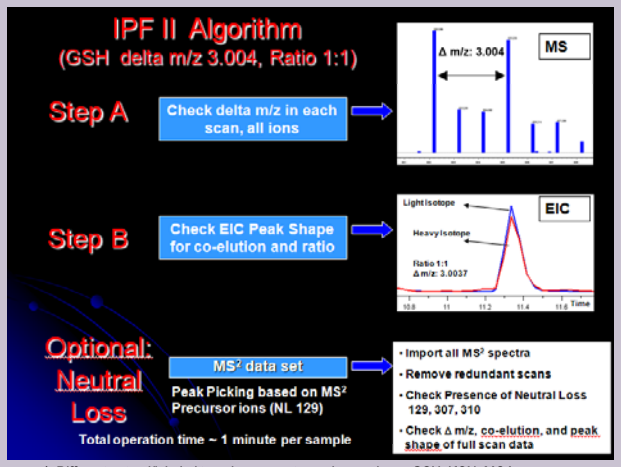
IPeaks , included in the MsXelerator™ software (MsMetrix), was developed to obtain a peak list of ions matching the isotopic pattern which at the same time possess a significant and real chromatographic peak shape. IPeaks greatly reduces the time needed for data exploration and processing. The output of IPeaks consists of a reconstructed TIC containing only the peaks of interest and a table listing all detected peaks matching the isotopic pattern.

Within the Isotopic Pattern Filtering algorithm (IPF) additional tools and methods are available to reduce the number of false positives; Mass Defect Filtering, Background Subtraction, Co-elution checks, Neutral Loss Checks (GSH assay), Spike removal and the possibility to perform Metabolite ID on all detected peaks, using common Biotrans rules.

Examples are presented from complex (in vivo) data matrices using $^{12}C/^{14}C$ radioactive labeling, GSH labeling assays (reactive metabolite profiling), Chlorine containing metabolites, and one example from a Metabolomics application ($^{14}N/^{15}N$ labeling), in which the isotopic pattern has variable mass differences, and depends on the number of nitrogen atoms in each compound. Samples were analyzed on Orbitrap, but all algorithms run at any mass resolution and can be applied to different data formats; Thermo, Waters, Bruker, Sciex, Agilent, mzXML

FLOW CHART OF IPF ANALYSIS USING GSH LABELING

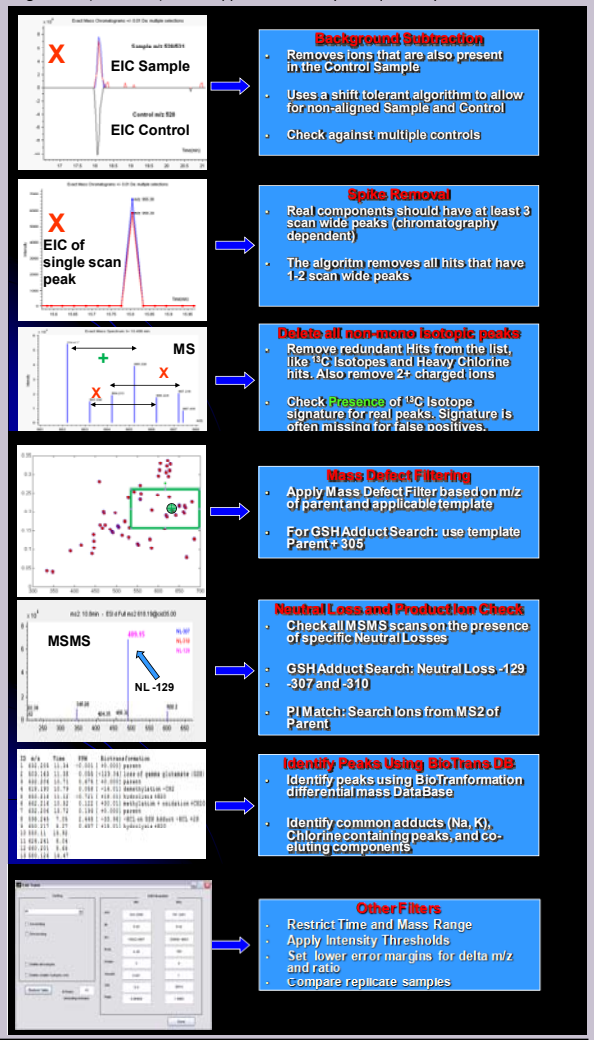
Detection of **Trapped Labeled Glutathione Adducts** is based on its specific labeling signature (Δ m/z 3.0037, ratio light/heavy 1:1). The **IPF II algorithm** is used to find all adducts in high resolution mode. The same algorithm can also be used to detect Chlorine containing metabolites, ^{13}C Isotopes, synthetically labeled drugs or any other isotope pattern.



* Different natural/labeled trapping reagents can be used, e.g. GSH, KCN, MOA

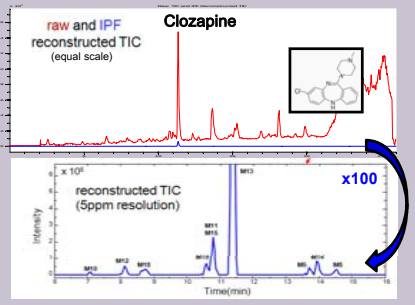
POST-ACQUISTION DATA MINING TOOLS

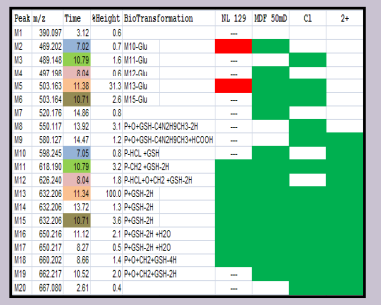
Although IPF is a highly selective algorithm, more selectivity is required to remove false positive and redundant hits from the initial results list. High Resolution Post Processing algorithms (see below) can be applied individually or sequentially:



EXAMPLES: ISOTOPE PATTERN FILTERING

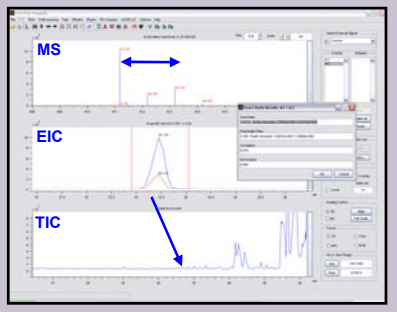
GSH/iso-GSH : Clozapine GSH Adducts + Post Processing. Isotopic Pattern: delta m/z = 3.0037, Ratio = 1:1





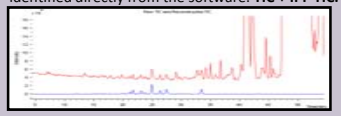
IPeaks results for GSH Adduct Search of Clozapine. **Left Panel** (a) Raw and Reconstructed TIC based on IPF Hits (checked against control sample), (b) Reconstructed TIC zoomed x100. **Right Panel:** Post Processing confirmation of Hits: Mass Defect, Neutral Loss, Presence of Chlorine and MetID results (green= pos. confirmation).

$^{12}C/^{14}C$ Pattern: SLV 13283 - In vivo detection of Metabolites in a Radioactive Assay:



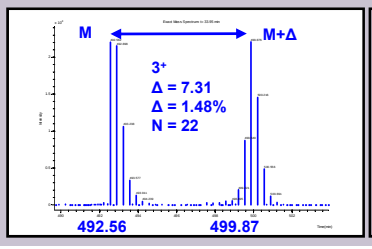
IPeaks was used to find metabolites in a complex in vivo drug *radioactive* screening assays.

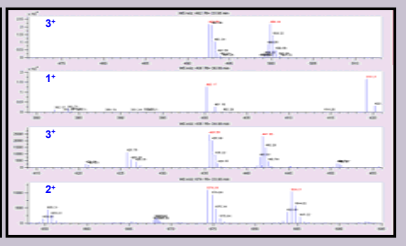
- Mass Difference $^{12}C/^{14}C$: 2.0032
- Ratio: determined from main metabolite in the data: R=3.4 (see inset). IPF allows accurate determination of metabolite isotopic pairs for ratios up to R=25. For larger ratios the co-elution check is inappropriate.
- 62 metabolites detected, of which 43 could be identified directly from the software. **TIC + IPF TIC.**



Metabolic Labeling: $^{14}N/^{15}N$: delta m/z = not known, Ratio = 1:1

In (Quant) Proteomics and Metabolomics, $^{14}N/^{15}N$ metabolic labeling is a common labeling strategy to detected up regulated or down regulated peptides and components. Without knowing the peptide sequence or structure, the mass difference between light and heavy isotope is unknown because the number of nitrogen's is not know. IPeaks uses a fast and ***accurate iterative algorithm*** to detect matching pairs. Additionally, for peptides we apply the 1.2% N rule; mass differences between light/heavy isotopes is 1.2% on average (Averagine Model):
Left Panel: Example Hit showing 3^+ ion containing 22 nitrogen atoms. **Right Panel:** Top Hits from experimental data.





The MsXelerator™ 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 detection and characterization of any compound having a specific isotopic signature in less than a minute. High Resolution Post Processing filters are used to increase selectivity and remove false positives. Neutral Loss Filtering, Mass Defect Filtering, Background subtraction and checking presence of ^{13}C isotope peak are all powerful features to support the nature of being true metabolites.