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

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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 false 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 possesses 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 Biomarkers rules.

Examples are presented from complex (in vivo) data matrices using 13C/13C radioactive labeling, GSH labeling assays (reactive metabolite profiling), Chlorine containing metabolites, and one example from a Metabolomics application (15N/15N 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. IPeaks 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.

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, 13C isotopes, synthetically labeled drugs or any other isotopic pattern.

IPF II Algorithm (GSH delta m/z 3.0037, Ratio 1:1)

**Step A**
- Check delta m/z in each scan, all ions
- Add: peak picking via mask
- Select ions with a peak picking of 5

**Step B**
- Check EIC peak shape for co-elution and ratio
- Select ions with a peak picking of 5
- Filter on retention time (RT) for MSMS
- Select ions with a peak picking of 5

Optional: Neutral Loss
- Select ions with a peak picking of 5
- Neutral loss analysis
- Select ions with a peak picking of 5

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 specific 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 13C isotope peak are all powerful features to support the nature of being true metabolites.

POST-ACQUISITION 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:

**Background Subtraction**
- Remove peaks that are not present in the Control Sample
- Check a suitable algorithm to allow for removal of co-eluting and non-specific ions
- Check against multiple controls

**Ratio Processing**
- Preprocess peaks that fail at least one standard deviation (SD) dependent
- To an algorithm removes all hits that fail 1SD/2SD/3SD

**Identify all tentative isotopic pattern peaks**
- Apply mass deficient/deficient ions to the control sample, single component, multiple components

**Neutral Loss and Peak Reduction**
- Check Mass and Mass Defect of the Peak
- Identity of Peaks using MS Trans DB
- Identity confirmation based on mass/charge ratio
- Identity common artifacts (e.g. adducts, isotope overlap, coeluting components)

**Spike RT and Mass Difference**
- Check MS and Mass Defect of the Candidate
- Apply ion intensity threshold
- MS and MS/MS ratios with high signal ratios

**Check All**
- Check all MS and MS/MS ratios
- Compare with the control sample

**Check All**
- Check all MS and MS/MS ratios
- Compare with the control sample

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EXAMPLES: ISOTOPE PATTERN FILTERING

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

Peaks results for GSH Adduct Screen of Clozapine: Left Panel (a) Raw and Reconstructed TIC based on IPI Hits (checked against control sample), (b) Reconstructed TIC zoomed ×100. Right Panel: Post Processing confirmation of Hits: Mass Defect, Neutral Loss, Presence of Chlorine and Metabolite results (green + pos. confirmation).

13C/13C 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 assay:
- Mass Difference 13C/13C: 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 + IPI TIC.

**Metabolic Labeling**: 14N/14N: delta m/z = not known, Ratio = 1:1

In (Quanti) Proteomics and Metabolomics, 14N/14N metabolite labeling is a common labeling strategy to detect 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 is not known. Peaks 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 (Average Modif) Model:

Left Panel: Example HS showing 3 ion containing 22 nitrogen atom. Right Panel: Top Hits from experimental data.

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