

MS-Xelerator™: Advanced Algorithms for LC/MS Data Processing applied to Impurity Profiling, Differential Analysis, Metabolite Profiling and Biomarker Discovery

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Introduction

The amount of data from LC/MS experiments, coming from Impurity Profiling, Degradation Profiling, Metabolite Profiling and Biomarker Discovery studies, has increased extremely.

In the case of **Impurity and Metabolite Profiling** often long processing times are necessary to find **significant and relevant** peaks in complex samples. Searching specific drug metabolites in samples containing thousands of peaks is a difficult and time-consuming task.

In the area of **Differential Analysis, Biomarker Discovery and Metabonomics** more advanced chemometric and bioinformatic techniques are needed to find specific peaks or combinations of peaks to discriminate between different classes of samples, e.g. healthy versus diseased. In general, multiple steps are necessary to solve these difficult problems (binning, alignment, peak picking and the choice of the proper classification algorithms). Due to many possible pitfalls, advanced knowledge of specialized algorithms and methods will be necessary in order to arrive at valid conclusions.

MS-Xelerator™ is a software program dedicated to all of the above tasks. It contains a large number of powerful modules and algorithms which will even help the inexperienced user to solve most of the above problems. MS-Xelerator contains the following modules:

Modules:

MBrowser: Powerful interactive graphical environment specialized for Impurity Profiling and in-depth analysis of single samples.

MPeaks: Fast and easy-to-use module for Peak Picking, Conversion of Peaks to Components, Differential Analysis and Metabolite Identification.

IPeaks: State of the art algorithms for finding components having specific Isotope Patterns (Cl, Br, labeled amino acids/drugs, user defined patterns).

MS Viewer: Specialized plots of mass chromatograms and mass spectra (overlay – matrix – line, in combination with external signals).

MS Compare: Comparison of series of LC/MS samples, Biomarker Discovery, PCA, Clustering, Data Mining and Explorative Search.

Features:

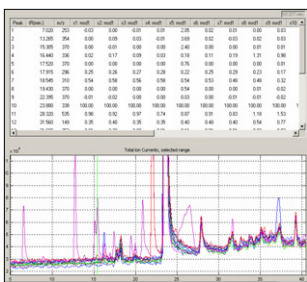
- Metabolite & Degradation Identification using Prediction Lists
- Co-correlation with external signals (UV, ELSD)
- Specific Impurity Profiling tasks
- High Res. Mass Defect Filters, Data Mining & Filter Modules
- Baseline Correction, De-Spiking, Chromatographic Alignment (MS ↔ UV)
- Search results based on predefined m/z list

Impurity Profiling: Drug Stress Testing & Batch Comparison

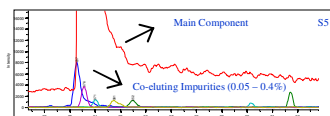
Data: 13 samples from stress testing study. Treatments: acid, base, moisture, oxidation, light, temperature, etc.

Goals: i). find and identify impurities in all samples simultaneously, ii). compare Samples/Batches

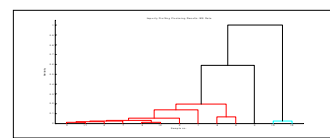
Workflow: 13 Samples -> Smooth -> De-Spike -> Align -> Create Table -> Visualize -> Cluster Samples
Total time needed to complete full analysis: about 10 min.



Result Table & processed TIC's (MsCompare)



5 Impurities detected under drug substance peak (MPeaks)



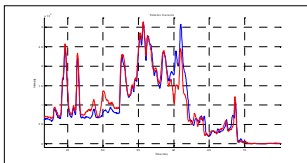
Sample Comparison using Clustering (MsCompare)

Differential Analysis

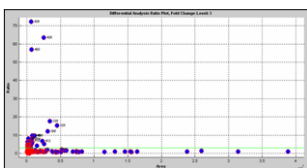
Data: treated and non-treated sample

Goal: find all peaks in sample A not found in sample B (fold change level: 3)

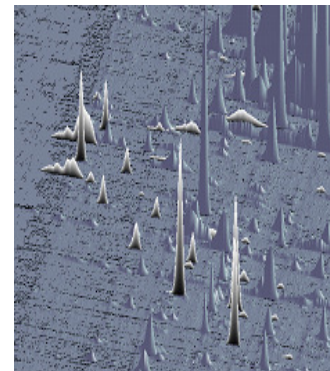
Workflow: 2 Samples -> MPeaks -> convert to components -> Differential Analysis -> Visualize
Total time needed to complete Differential Analysis: about 20 seconds.



Overlay of Total Ion Currents (MsCompare)

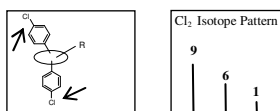


Differential Analysis: Area - Ratio Plot (MPeaks)



3D LC/MS Plot showing marked differential peaks

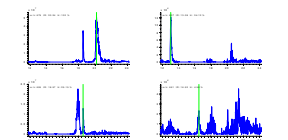
Metabolite Profiling of Drugs containing Chlorine



MPeaks -> 731 peaks (7 sec.)
IPeaks -> 16 peaks (24 sec.)



Raw TIC and IPeaks reconstructed TIC, rat bile 24h



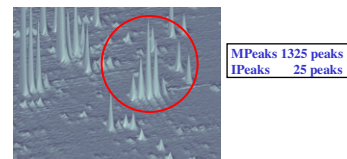
Detected peaks containing Cl₂ (IPeaks - MsViewer)

SITE: Stable Isotope Tagging of MHC Class I Epitopes

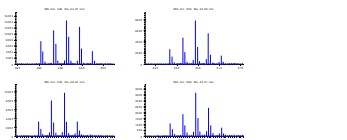
Data: 1 sample -> labeled amino acids (³H₁-Leucine)

Goal: find peptides having unique Isotope Pattern

Workflow: 1 Sample -> IPeaks -> Visualize
Total time needed to complete analysis: about 30 sec.



Isotope Pattern: Binomial Distribution, Δ m/z 3

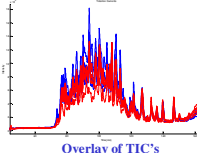


Mass Spectra of detected MHC class I epitopes

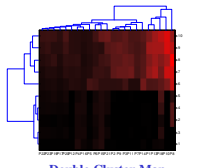
Biomarker Discovery

Data: urine samples of controls and patients (5/5)

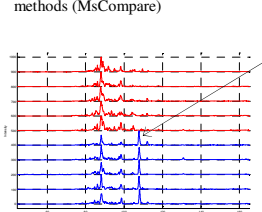
Goal: find unique peaks discriminating both classes using non-peak-detection methods (MsCompare)



Overlay of TIC's



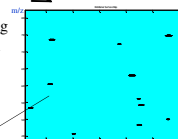
Double Cluster Map (Samples & Peaks)



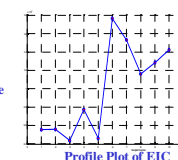
Extracted Ion Current at unique m/z value



Principal Component Analysis (PCA)



BioMarker Surface Map of aligned and unique peaks



Profile Plot of EIC

MS-Xelerator has been designed to be extremely fast, easy-to-use and is independent of instrument vendor. The software offers a multitude of algorithms and modules to solve a large number of complex problems in LC/MS Data Processing and Profiling studies.