Interactive Ion Peak Analysis and Differencing for Comparing Multidimensional Chromatography Data

Chase Heble;[1] Daniel Geschwender;[1] Qingping Tao;[1]
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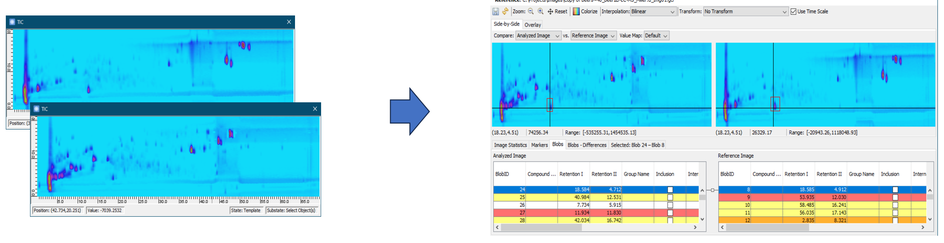
Introduction

Comprehensive two-dimensional chromatography offers superior separation capabilities for complex mixtures, but the resulting data complexity necessitates advanced comparative analysis methods. A common scenario involves comparing two samples to identify similarities and differences. We demonstrate our methods through two applications including performing new peak detection (NPD) **[1]** for LC-MS data of peptide samples, and identifying and comparing common and unique compounds from pairs of GCxGC-MS chromatograms.

Interactive Comparative Visualization and Differencing

We use comparative visualization methods **[2]** built upon conventional image comparison techniques

- Align two chromatograms and apply specialized color maps to enable visual identification of discrepancies
- Our interactive side-by-side differencing tool **[3]** enables the matching of peaks across two chromatograms using chromatographic retention times
- Gives both qualitative and quantitative analysis of sample differences at the individual peak level



Our existing interactive side-by-side differencing tool

Comparative Visualization and Differencing of Ion Peaks

Ion Peak Detection
- Peak detection in individual ion chromatograms
- Combining peaks of the same analyte across multiple ions

Targeted Ion Peak Detection
- Ion peaks in one chromatogram used to target detection in other
- 1-to-1 mapping of detections and recovery of peaks missed or filtered in the initial detection

Ion Peak Matching
- Ion peaks matched using RT location and spectra criteria
- Matches must be bidirectional
- Ensures correspondence is established between matches

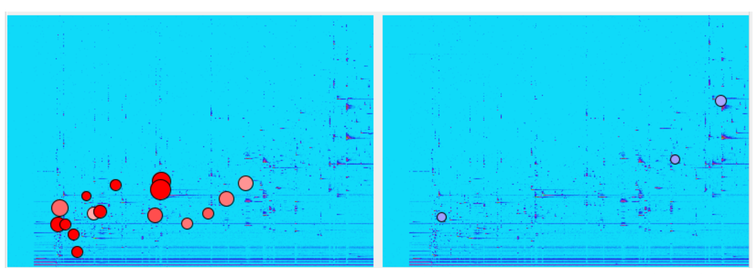
Interactive Visualization and Differencing
- Side-by-side display (TIC, SIC, ion maps) of chromatograms with peaks displayed
- Peak tables and match tables for quantitative differencing with filtering tools

Approach 1: Targeted Ion Peak Detection

- Targeted detection with 5ppm ion m/z tolerance
- A fold change of >5 was used to perform new peak detection (NPD) **[1]**
- 2D visualizations generated using a RT x MS data view

Data Set
- LC-MS data of the NISTmAb RM 8671 from the Multi-Attribute Method (MAM) Consortium Interlaboratory Study [4]
- From the data set, we focused on data from a single participant (participant 16)

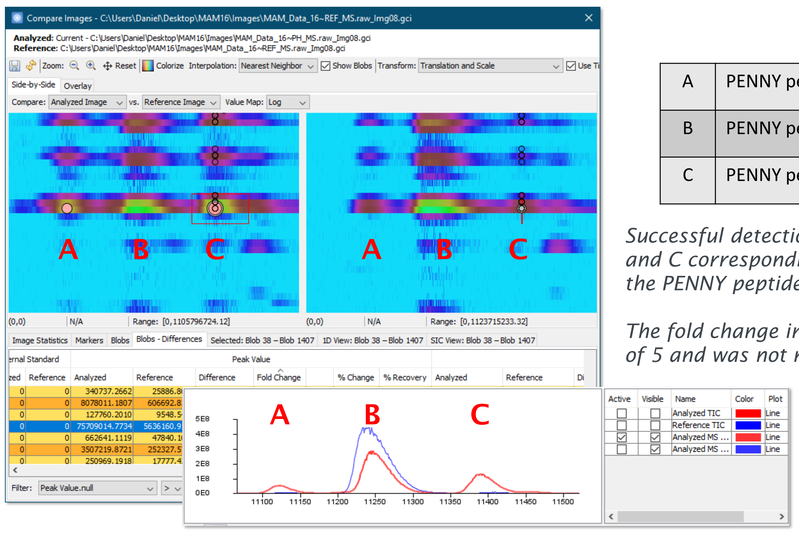
Spiked vs Reference:
- Comparing the spiked reference to the original reference sample
- Successfully found all 15 expected calibrants as new peak detections in spiked
- Found 3 decreased peaks and 1 additional new peak



new/increased peaks in spiked sample overlayed on spiked

missing/decreased peaks in spiked sample overlayed on reference

pH vs Reference:
- Comparing reference subjected to pH stress to original reference sample
- Found all NPD reported by >50% of study participants
- Found total of 173 new peak and 53 missing peak candidates



A	PENNY peptide - Deamidation (early peak)
B	PENNY peptide - original
C	PENNY peptide - Deamidation (late peak)

Successful detection of the new/increased peaks A and C corresponding to the deamidation species of the PENNY peptide (B).

The fold change in peak B fell below the threshold of 5 and was not reported as a decreased peak.

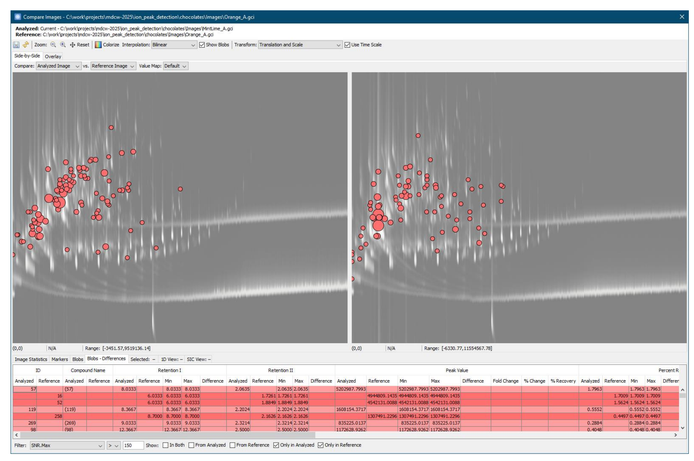
Approach 2: Ion Peak Matching

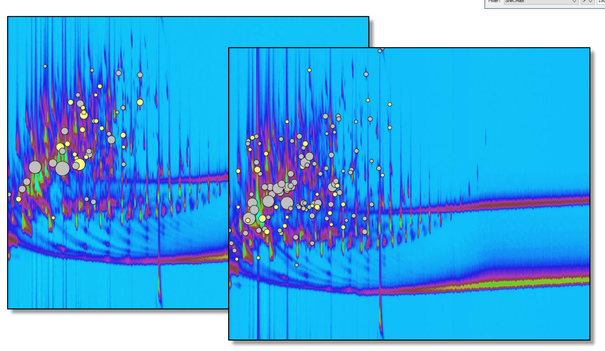
- Find bidirectional matching between detections in both chromatograms
- Matching criteria: matched apex within RT tolerance, matching detection ion m/z or spectral match of >750

Data Set
- GCxGC-TOFMS public data set of different dark chocolates [5]. Mint-Lime and Orange flavors are used for this demonstration. The figures presented all have Mint-Lime on the left and Orange on the right.
- JEOL AccuTof GC+ mass spectrometer with an Agilent 7890 GC.

Unique Peaks Filter
Significant unique ion peaks are shown using filters:
- 'Unmatched' status
- SNR > 150

The figure to the right shows the filtered peaks on the 2D chromatogram and table.

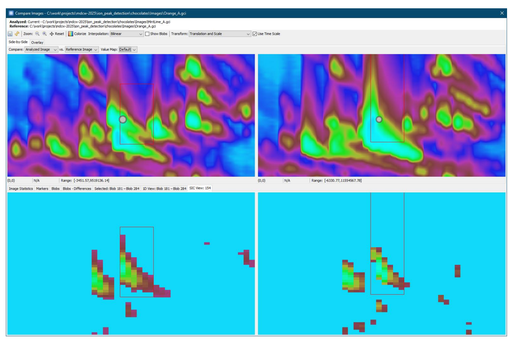




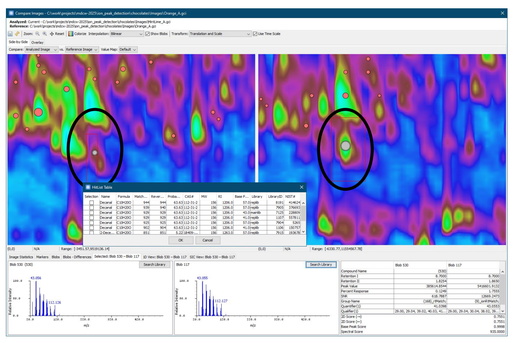
Common Peaks Filter
Common ion peaks are shown are shown with the 'In Both' filter.

The figure to the left shows common peaks that have a significant response difference. Filtering was done using > +200% change and < -50% change.

Peak Comparison
Common compounds are matched and allow comparison between the images. The compound response values, properties, and spectra can be viewed for both samples.



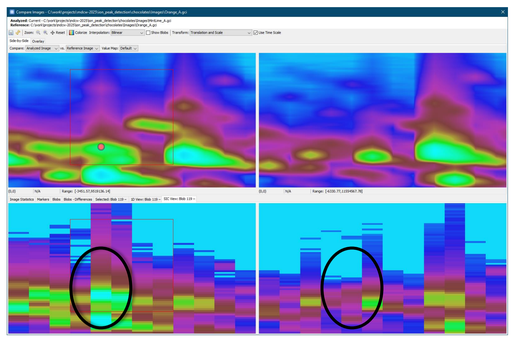
The ion peak detection and matching highlights the deconvolved Eucalyptol peak, which has a higher response in Mint-Lime.



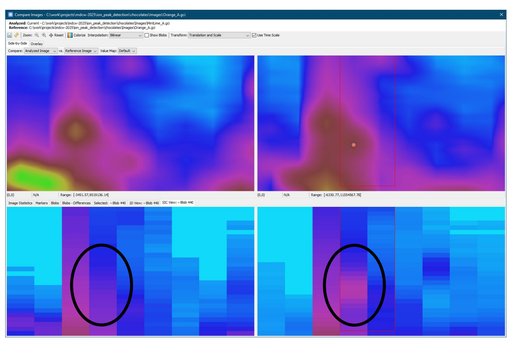
Decanal is present in both samples, but more significant in Orange.

Unique compounds are viewed and identified, with the SIC view used to analyze the alternate chromatogram at the RT location and ion ranges.

The figures below highlight a unique compound in each sample.



Ion peak compound unique to Mint-Lime, with SIC view. Library search returns 'Citral' as a likely match.



Compound unique to Orange, with SIC view. Library search showed several good matches for compounds with the formula C10H16.

References

1. R. Rogers et al., MS in QC: A Single Multi-attribute Method for Quality Control and Release Testing of Biologics. Presented at CASSS MS 2013, Boston, September 24 2013.
2. B. Hollingsworth et al., Comparative Visualization for Comprehensive Two-Dimensional Gas Chromatography. J Chromatogr A. 2006 Feb 10;1105(1-2):51-8.
3. Q. Tao et al., New Peak-Based Differencing Tools for Side-by-Side Comparison of Two Samples with GCxGC-MS. GCxGC Symposium, May 2018.
4. T. Mouchahoir et al., New Peak Detection Performance Metrics from the MAM Consortium Interlaboratory Study. J. Am. Soc. Mass Spectrom. 2021, 32, 4, 913–928.
5. B. Weggler et al., 2020, "Benchmark GCxGC Data, Chocolate", https://doi.org/10.7910/DVN/AKT6BH, Harvard Dataverse, V1.

Data processes and screenshots for this publication are from an alpha version of MDC Investigator v2025
(Visit www.gcimage.com for current v2024 releases)

GC Image
Software for Multidimensional Chromatography

Interactive Ion Peak Analysis and Differencing for Comparing Two-Dimensional Liquid Chromatography Data

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Introduction

Identifying chemical variations between samples is crucial for various applications, including process monitoring, sample classification, and correlative studies. A common scenario involves comparing two samples to identify similarities and differences. We demonstrate our methods through two applications including performing new peak detection (NPD) **[1]** for LC-MS data of peptide samples, and identifying and comparing common and unique compounds from pairs of LCxLC-MS chromatograms.

Interactive Comparative Visualization and Differencing

We use comparative visualization methods **[2]** built upon conventional image comparison techniques

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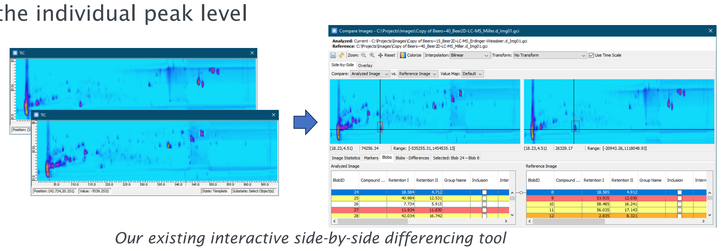
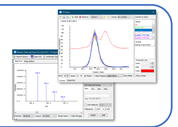
- Gives both qualitative and quantitative analysis of sample differences at the individual peak level



Our existing interactive side-by-side differencing tool

Comparative Visualization and Differencing of Ion Peaks

Initial Ion Peak Detection
- Peak detection in individual ion chromatograms
- Combining peaks corresponding to the same analyte across multiple ions



Targeted Ion Peak Detection
- Ion peaks in one chromatogram used to target detection in other
- Ensures 1-to-1 mapping of detections
- Won't miss peak due to initial detection settings

Ion Peak Matching
- Ion peak in one chromatogram matched to peaks in the other
- Matching criteria based on RT location and spectra
- Matches must be bidirectional
- Ensures correspondence is established between matches

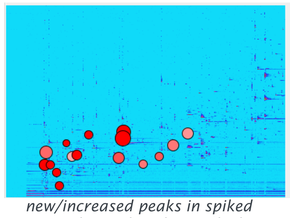
Interactive Visualization and Differencing
- Side-by-side display (TIC, SIC, ion maps) of chromatograms with detections overlayed
- Match table for quantitative differencing

Approach 1: Targeted Ion Peak Detection

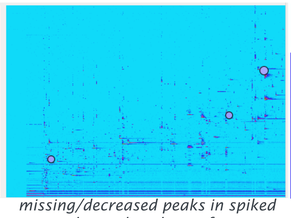
- Detections in one chromatogram used for targeted detection in other
- Search detection ion m/z within 5ppm and within peak RT bounds
- Find max response location and report fold change
- >5 absolute fold change reported as a NPD
- Verified by comparing ion peak maps for both chromatograms

Data Set
- LC-MS data from the Multi-Attribute Method (MAM) Consortium Interlaboratory Study **[4].**
- We focused on data from one participant: various runs of samples containing peptides. (reference, spiked, pH stressed)
- Instrument information unavailable due to study anonymization

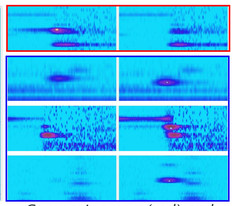
Spiked vs Reference:
- Comparing reference sample spiked with 15 calibrant peptides to original reference sample
- Successfully found all 15 calibrants as new peak detections in spiked
- Additionally, found 1 more new peak and 3 more missing peaks



new/increased peaks in spiked sample overlayed on spiked

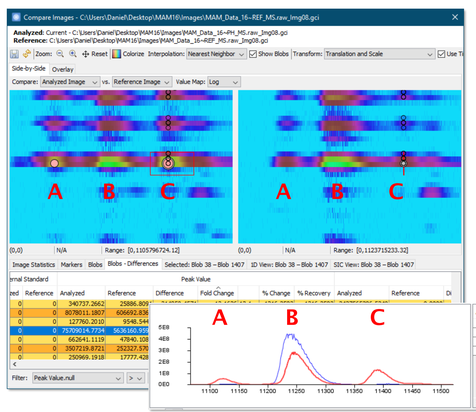


missing/decreased peaks in spiked sample overlayed on reference



Comparing new (red) and missing (blue) peaks across samples

pH vs Reference:
- Comparing reference subjected to pH stress to original reference sample
- Found all NPD reported by >50% of study participants
- Found total of 173 new peak and 53 missing peak candidates



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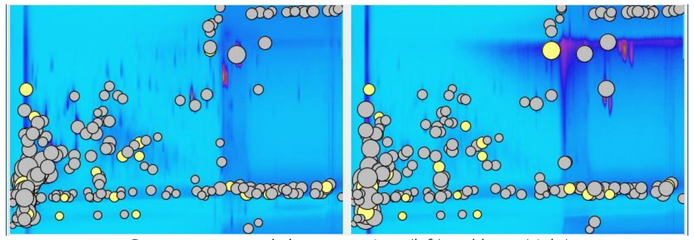
The fold change in peak B fell below the threshold of 5 and was not reported as a decreased peak.

Approach 2: Ion Peak Matching

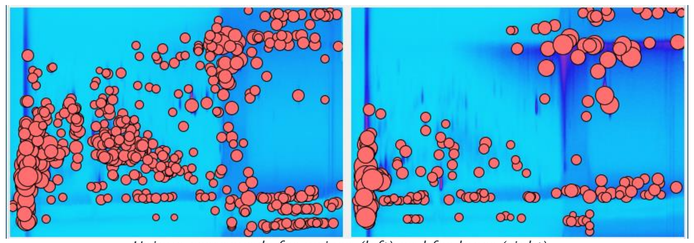
- Find bidirectional matching between detections in both chromatograms
- Matching criteria: matched apex within peak RT bounds, detection ion m/z within 100 ppm or spectral match of >750

Data Set
- LCxLC-MS data from application note on Fingerprinting Analysis of Different Types of Beer **[5].**
- We consider two beer samples: 1 German weizen, 1 American lager
- Agilent 1290 Infinity 2D-LC solution coupled with an Agilent 6530 Accurate-Mass Q-TOF LC/MS system

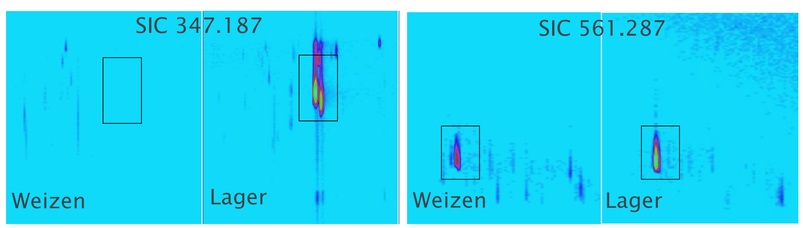
Weizen vs Lager:
- Comparing German weizen beer to American lager beer
- 203 common peaks, 511 unique to weizen, 200 unique to lager
- Found difference in presence of iso-α-acids (in weizen) and reduced iso-α-acids (in lager) consistent with expectation due to regulations in Germany



Common compounds between weizen (left) and lager (right)



Unique compounds for weizen (left) and for lager (right)



Verifying matches by checking the SIC of the detection ion. Left is a unique detection for lager: the reduced iso-α-acids. Right is a common detection, but somewhat more abundant (1.4x) for lager.

References

1. R. Rogers et al., MS in QC: A Single Multi-attribute Method for Quality Control and Release Testing of Biologics. Presented at CASSS MS 2013, Boston, September 24 2013..
2. B. Hollingsworth et al., Comparative Visualization for Comprehensive Two-Dimensional Gas Chromatography. J Chromatogr A. 2006 Feb 10;1105(1-2):51-8.
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