Data Analysis Workflow for Comprehensive Two-Dimensional Liquid Chromatography (LCxLC)

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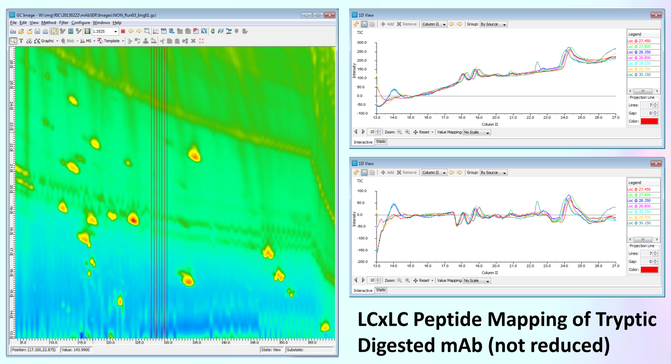
The **End-to-End Data Analysis Workflow (E2E)** supports comprehensive comparative chemical analysis with comprehensive two-dimensional liquid chromatography (LCxLC). Comprehensive comparative analysis requires evaluation of every constituent in every sample and is the most general problem of analytical chemistry. LCxLC is a powerful emerging technology that produces data that is rich with chemical information, but distilling that information from the detector data involves a sequence of data processing challenges. E2E utilizes robust peak-region features and encompasses three principal steps: (1) Chromatogram Processing, (2) Feature Extraction, and (3) Comparative Analysis. E2E is implemented in GC Image® LCxLC software with Image Investigator™.

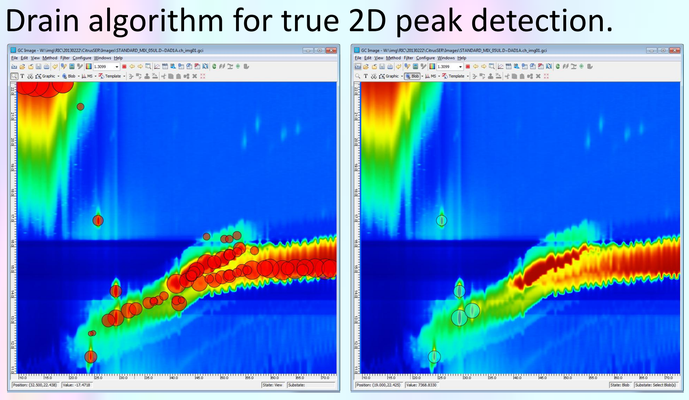
Chromatogram Processing

1. **Preprocessing.**
   In each chromatogram:
   a) Align the data to the modulation cycle.
   b) Correct the baseline to zero mean.



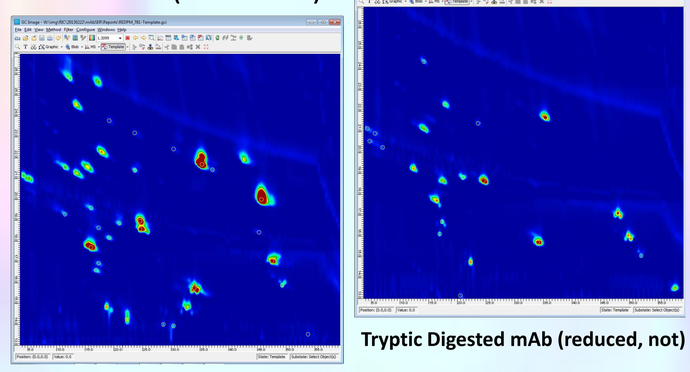
LCxLC Peptide Mapping of Tryptic Digested mAb (not reduced)

2. **Peak detection.**
   In each chromatogram, detect each peak using the Drain algorithm for true 2D peak detection.



Standards mix: Coumarin, Trioxsalen, 5-MOP, Psoralen, & 8-MOP

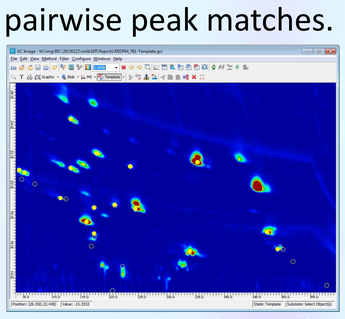
3. **Template building.**
   For each chromatogram, record the retention times, spectrum (if applicable), and CLIC matching constraints (if desired).



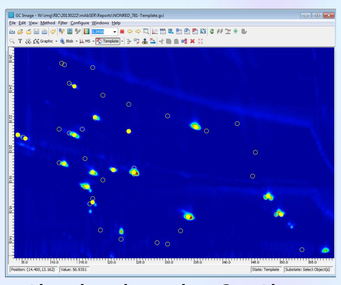
Tryptic Digested mAb (reduced, not)

Feature Extraction

4. **Template matching.**
   Match each template to every other chromatogram and record the pairwise peak matches.

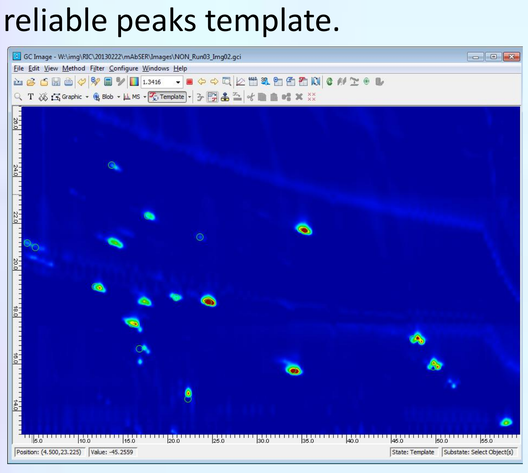


mAb not reduced template & mAb reduced target



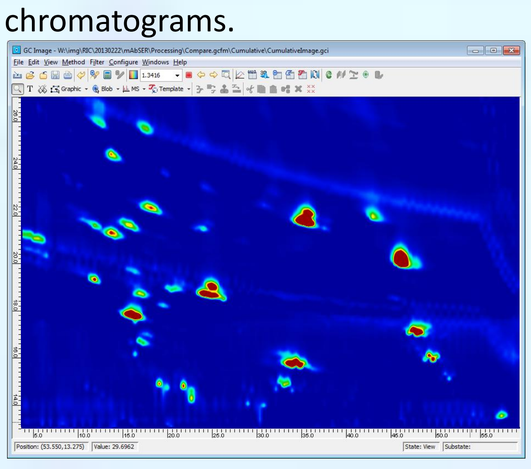
mAb reduced template & mAb not reduced target

5. **Reliable peaks selection.**
   From the pairwise peak matches, record the peaks that are matched reliably across chromatograms in a reliable peaks template.



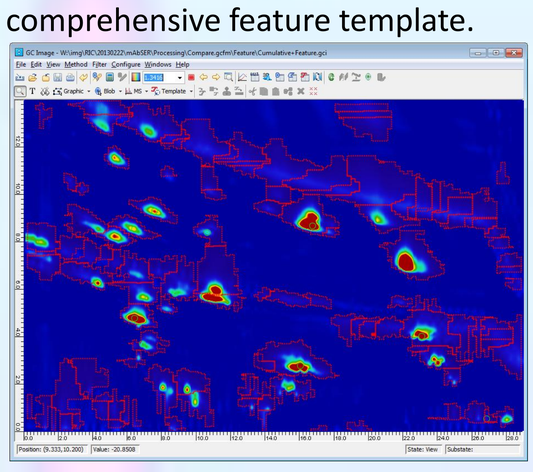
mAb not reduced & reliable peaks template

6. **Composite chromatogram.**
   Align every chromatogram using the reliable peaks template. Then, create a composite chromatogram with the data from the aligned chromatograms.



mAb reduced & not reduced composite

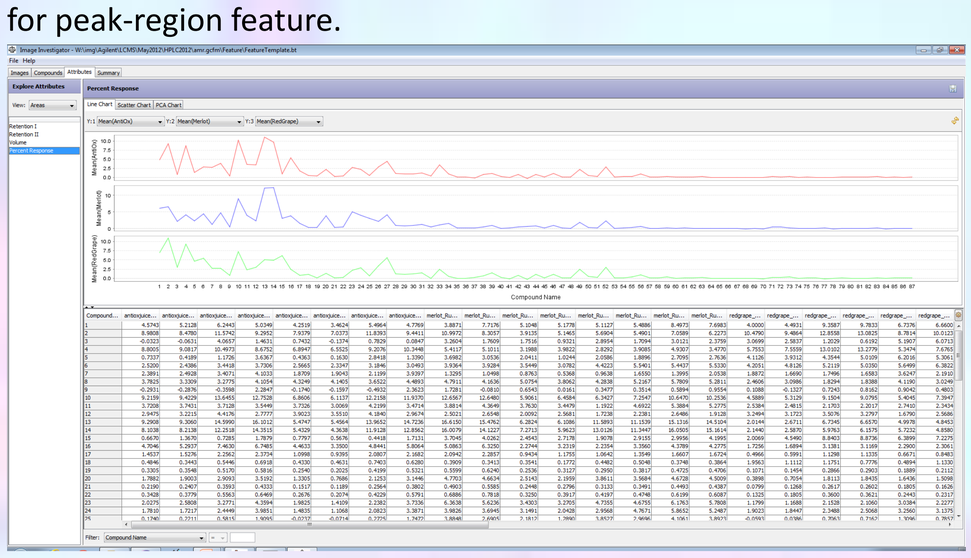
7. **Feature template.**
   Detect peaks in the composite chromatogram. Then, define a region from the footprint of each peak. Combine these peak-regions with the reliable peaks to create a comprehensive feature template.



mAb composite with marker peaks & peak-regions

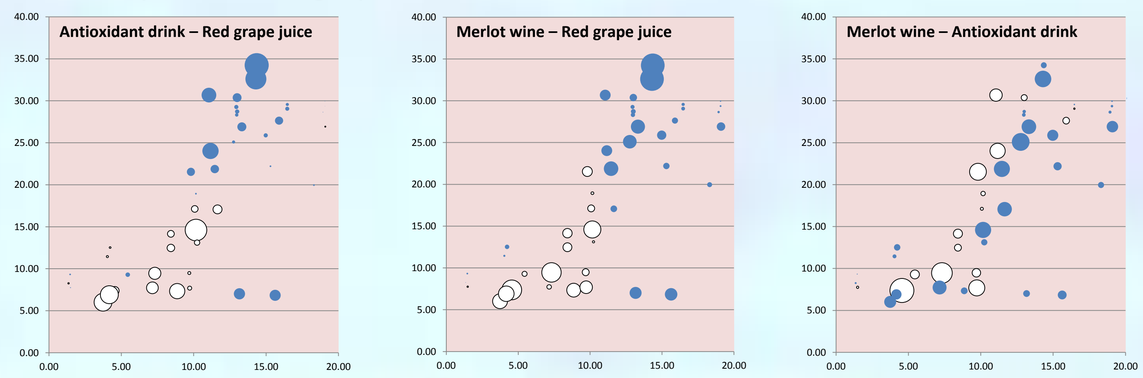
Comparative Analysis

9. **Chromatogram-feature analysis.**
   Match and apply the feature template to each chromatogram. The result is a Chromatogram-Feature Table with a column for each chromatogram and a row for peak-region feature.



Chromatogram-Feature Table with peak-region features for 24 chromatograms of three anti-oxidant beverages.

10. **Comparative feature analysis.**
    Analyze the Chromatogram-Feature Table for important differences, anomalies, trends, patterns, fingerprints, clusters, classifications, or chemical markers.



Compositional differences between three anti-oxidant beverages: a commercial anti-oxidant drink, red grape juice, and Merlot wine. Circles are located by peak-region retention times. Blue circles indicate that the first average (two replicates of four different concentrations for a total of eight chromatograms) is greater than the second average. White circles indicate that the second average is greater than the first average. Circle areas indicate the magnitudes of the differences. Merlot has the largest relative concentration of preferred anti-oxidants (upper-right), such as resveratrol, and red grape juice has the least.

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