METHOD DEVELOPMENT AND AUTOMATION FOR COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY (LCxLC)

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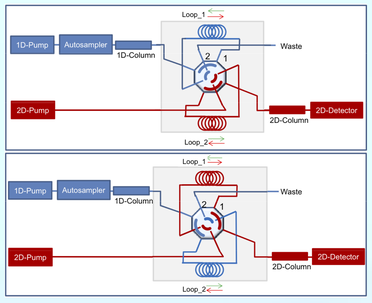
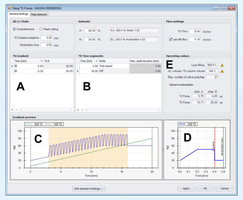
Chromatography

LCxLC employs a system of pumps and valves to control the chromatography.



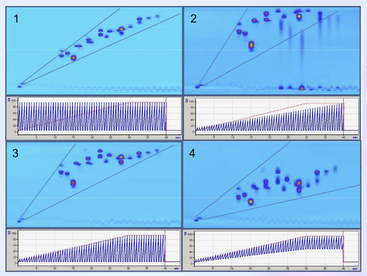
LCxLC with a two-position, four-port duo-valve.
↔ Fill Direction
↔Analyze Direction
Top: Loop 1 filled, Loop 2 analyzed.
Bottom: Loop 1 analyzed, Loop 2 filled.

Sophisticated GUI interfaces provide flexible, interactive configurations of chromatographic settings.



A. Nested table for gradient.
B. Time & peak-based triggers.
C. Interactive graph of 2D gradient (to/from A & B).
D. Graph of single, repeated gradient snip.
E. Calculations based on settings.

Method development optimizes chemical resolution and use of the separation space.



In the graphs:
Red lines show the first-dimension gradient.
Blue lines show the repeating second-dimension gradient.
In the images, the angled lines show the use of the separation space.

1. The second-dimension gradient repeats between 5% and 95% organic.
2. Lower, progressively increasing organic in the second-dimension increases polarity separation.
3. Adjustment of the second-dimension gradient to change with the first-dimension gradient improves separations.
4. Increased organic composition in the second dimension increases peak capacity.

Data Processing

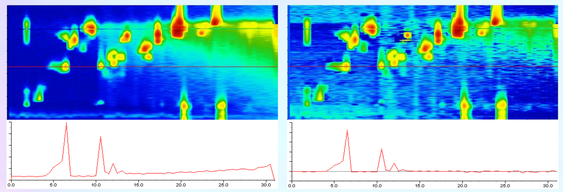
Automation of data processing requires configurations for baseline correction, peak detection, and peak identification.

Experimental Samples & Chromatography

Polyphenols mixture. MS Configuration:: column 1: 1.0x150mm at 0.05 mL/Min, modulation: 30Sec/Separation, column 2: 2.1x50mm at 1 mL/Min, ESI-TOF-HRMS: 30 Spectra/Sec.

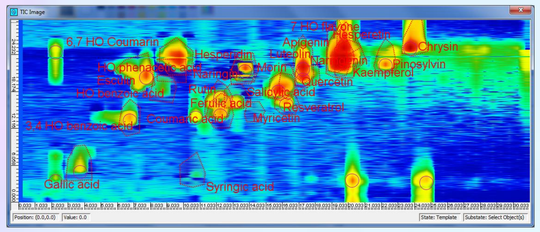
Baseline Correction

Left: Before baseline correction, a positive-valued baseline increases during the course of the run, as illustrated in the 1D trace through the center of the second-column separations.
Right: After 2D gradient baseline correction, the baseline is near zero and steady during the course of the run



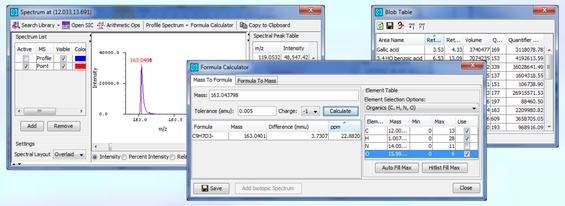
Peak Detection & Template Matching

A template records a pattern of possible peaks with their identifying characteristics, such as MS signature, and other analytical structures such as 2D retention-time regions. Template matching uses 2D pattern recognition to align a pattern with the detected peaks, thereby identifying them.



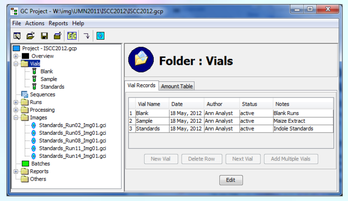
High-Resolution Mass Spectrometry

HRMS provides accurate mass measurements for compound identification, isotopic ratios, and selected ion quantification.



Data Management

Data management encompasses raw and processed chromatograms, software methods and configuration files, amount and calibration tables, and other metadata.

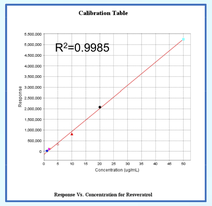


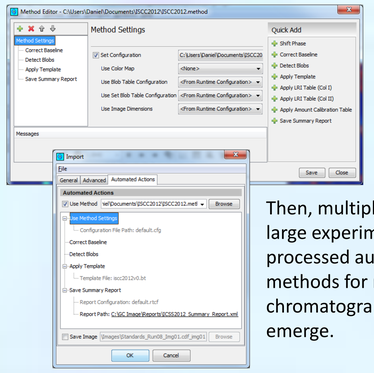
Projects

Projects may involve the handling of many chromatograms from blank runs, standards runs, and samples.

Calibration & Batches

Calibration requires the collection and processing of data from multiple runs as well as ancillary information such as amount tables. Other operations, such as QA/QC similarly may require batches of multiple runs.





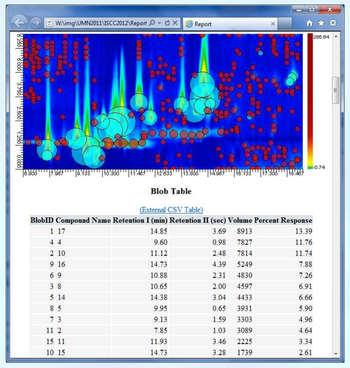
Methods

Automated data analysis methods record the sequence of operations and reporting.

Then, multiple samples, even from large experiments, can be processed automatically. Standard methods for multi-dimensional chromatography are beginning to emerge.

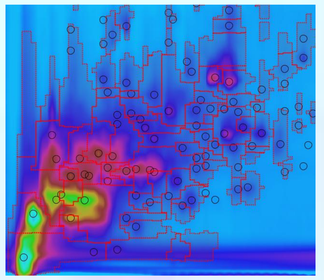
Reporting

Automation requires the generation of standard and custom reports. Some reports may collate results from multiple chromatograms and provide various components, such as images, tables, and graphs. (Indole data courtesy P. Carr, U. Minnesota.)



Informatics

LCxLC applications may entail targeted or non-targeted analyses. Goals of non-targeted analyses include sample classification, chemical fingerprinting, monitoring, sample clustering, and chemical marker discovery.

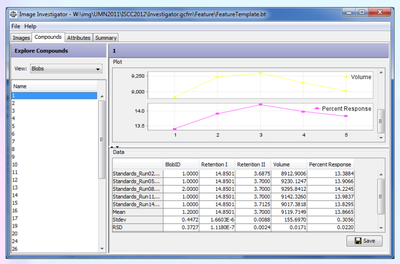


Features

Peak, region, and advanced peak-region features can be matched, aligned, and compared for cross-sample analyses. (Urine data courtesy P. Carr & D. Stoll, U. Minnesota.)

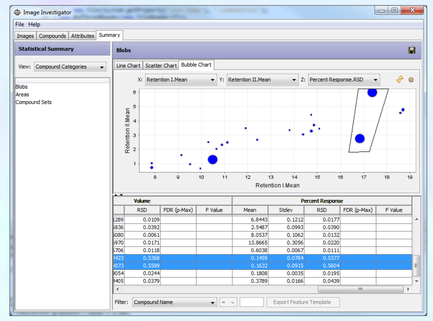
Statistics

Statistics can be viewed by image, feature, or attribute (RT, response, etc.). Tables and graphs visualize comparisons and trends.



Statistical Pattern Recognition

Pattern recognition techniques such as Fisher Ratio, Principal Component Analysis (PCA), K-Means, and Hierarchical Clustering support feature selection, clustering, classification, and chemical marker discovery. Interactive visualizations connect statistical analyses to the chemical separation space.



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GC IMAGE Software for Multidimensional Chromatography
Agilent Technologies