Development of Optimization Strategies for Multiple Heart-Cut Two-Dimensional Liquid Chromatography

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Introduction

Multiple heart-cut 2D liquid chromatography (mLC-LC) allows for the on-line analysis of several fractions of interest from the first-dimension (^{1}D) effluent by a second-dimension (^{2}D) separation [1]. With the ability to temporarily store fractions prior to ^{2}D analysis, mLC-LC yields additional separation power ideal for purity assessments and quantitation of main components in a sample [2].

Challenges Reducing Efficiency and Effectiveness

- **Sub-optimal fraction selection.** During characterization other co-eluting fractions may be present. Quantification: how often to fraction to allow for accurate quantification?
- **Time-consuming method development.** Currently, the operator must optimize the ^{1}D and ^{2}D methods individually, whereas their optima greatly depend on the composition, selection and number of fractions.

Improving the accessibility of multiple heart-cut chromatography

We present an optimization algorithm that automatically develops a sample-tailored method.

- **All Important Information from Your Sample.** Using chemometric strategies, the algorithm automatically selects regions of interest in the chromatogram and optimally utilizes the available loops.
- **Rapid, optimal method development.** The algorithm automatically optimizes the ^{1}D, the number of cuts per peak, and ^{2}D methods to maximize quantification.

Experimental

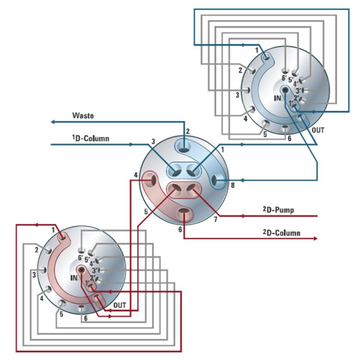
System: vial sampler, two high speed pumps, two diode array detectors and a multiple heart-cut valve equipped with 2 loop decks of 6x40 µl (figure on the right) from the Agilent 1290 Infinity II series.

^{1}D parameters: PL-SAX (1.8 µm dp, 50x4.6 mm, Agilent) column, a flowrate of 1.2 mL/min and a 2 µl injection volume. The mobile phase was 50/50 acetonitrile/ deionized water with a pH of 7.5 and 50/50 acetonitrile/ deionized water with 0.1M ammonium sulphate at a pH of 7.5.

^{2}D parameters: C18 ZORBAX RRHT Eclipse Plus (8 µm dp, 150x2.1 mm, Agilent) column and a flowrate of 0.2 mL/min. The mobile phase was deionized water with 10 mM tetramethyl ammonium hydroxide and acetonitrile.

Sample: mixture of ~15 dyes (~180 ppm each dye)

The algorithm was written using MATLAB 2019a (MathWorks, Natick, MA, U.S.A.) for the in-house 'multivariate optimization and refinement program for efficient analysis of key separations' (MOREPEAKS, https://www.morepeaks.org).



Schematic overview of a multiple heart-cut valve setup [3].

Optimization strategy

Step 1: ^{1}D Optimization

Aim: Pareto optimization of the ^{1}D to maximize the resolution score and to decrease the complexity of the fractions that will be further analyzed in the ^{2}D.

Applies principles of scanning-gradient experiments to scan the sample using two input chromatograms [4].

Step 2: Cut selection

Aim: Determine which fractions/peaks need to be transferred to the second dimension.

Preprocessing

- Smoothening: Savitzky-Golay
- Background correction: Asymmetric Least Squares

Peak detection

Deconvolution strategy of choice applied to scan chromatogram for co-eluting peaks. Curve-fitting strategy allows estimation of convoluted ^{1}D peak shape.

Characterization

Aim: Maximizing information yield from sample. User can include and exclude peaks. The program adds fraction regions from high to low priority, based on peak features and degree of overlap of the peak.

Quantitation

Aim: accurate quantification of peaks of interest. The program fractions the entire peak volume with additional pre- and superseding cuts of the user specified peak(s). Recovery can be assessed and predicted to optimize quantification accuracy.

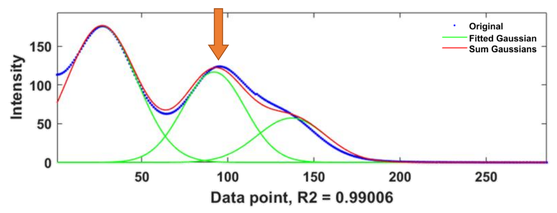
Step 3: ^{2}D Optimization

Aim: Optimization of all ^{2}D gradient programs through retention modelling prediction for all cuts.

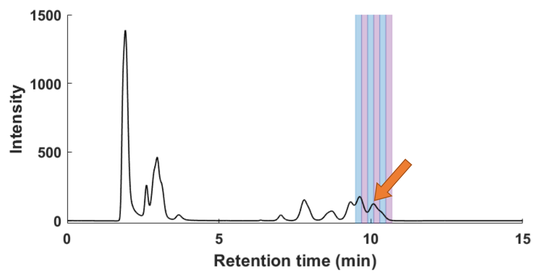
Applies principles of scanning-gradient experiments to scan the sample [4] using two heart-cut measurements with the same ^{1}D conditions and fractions.

Quantification Mode

1. User specifies peak(s) of interest
2. Deconvolution

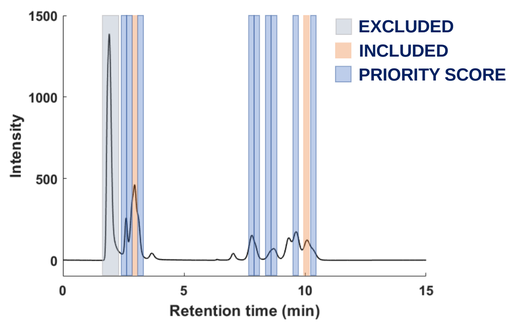


3. Fraction peak volume with preceding and superseding fractions



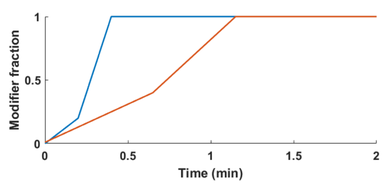
Characterization Mode

1. User can include and exclude peaks or specify a cut time
2. Cuts will be made based on a priority system
   I. Included peaks
   II. Priority score (peak features and overlap)
3. Algorithm considers technical limits when selecting cut times (e.g. loop availability)

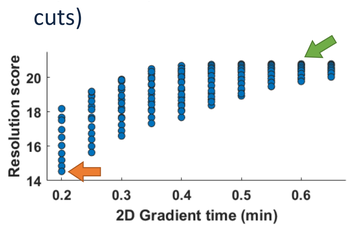


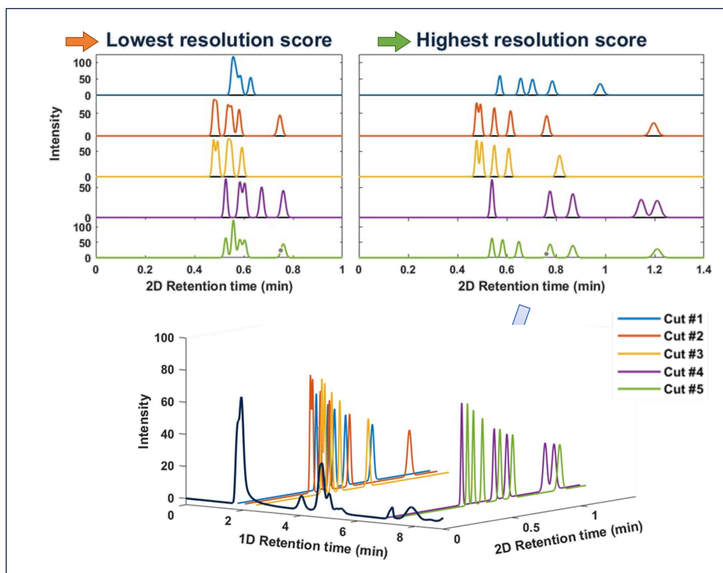
^{2}D Gradient Optimization

1. Algorithm simulates gradient programs for a range of method parameters and predicts elution across all fractions.



2. All methods assessed with resolution score (summarized separation performance of all cuts)





This figure shows the original ^{1}D chromatogram with the optimized ^{2}D chromatograms of all cuts at their respective ^{1}D time.

Conclusions

- Automated heart-cut 2D-LC optimization algorithm presented.
- Deconvolution strategies to identify and prioritize regions for heart-cut fractioning for quantification or characterization.
- Algorithm considers knowledge on instrumentation limits to allow optimal use of fraction loops
- ^{1}D and ^{2}D methods automatically optimized using initial scanning-gradient experiments

Future Perspectives

- Investigation of the influence of the cut times relative to the peak retention time on second dimension recovery.
- Use of shifting gradients to allow for more cut-independent gradient optimization.
- Assess the performance of the practical applicability of the current optimization strategy.

References & Acknowledgements

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Acknowledgements: The authors gratefully acknowledge support from the Agilent University Relations grant.

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