Evaluation of ACD/Autochrom Software for LC Method Development

Dow AgroSciences

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Background

Goal: Minimize time needed to optimize methods

Ensure selectivity, robustness, minimum measurement time attained by optimizing more than one method per project

Compare Advanced Chemistry Development's Autochrom software to offline optimization software

LCSimulator (method optimization) alone with manual importing of data

Autochrom includes instrument control, automated peak identification, and method optimization

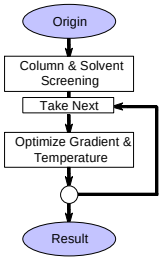
Evaluated using agricultural chemicals

Agilent 1290 Instrument

Development system to allow choosing 6 different columns, 10 different A or B mobile phases

Binary pump/DAD/6130 single quad MS

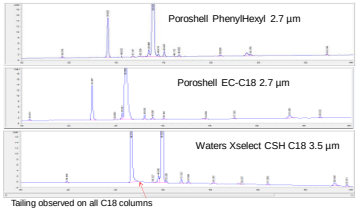
Experimental Design



Screening Data

Data collected with three columns and three solvents

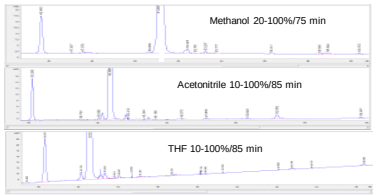
Example data comparing three columns using acetonitrile.



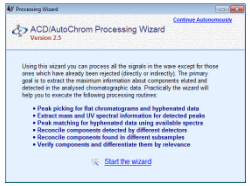
0.5 mL/min
A: 0.1% formic acid/water
B: 0.1% FA/solvent
40°C
5-100% acetonitrile/90 min
100x3 mm column size

Tailing observed on all C18 columns

Comparison of three strong solvents using the Poroshell PhenylHexyl column



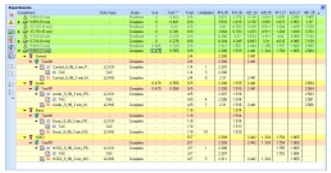
Data Processing



Goal of data processing: Every peak identified in every chromatogram
This is vital for optimization step.
Not necessary for every peak to be identified during screening

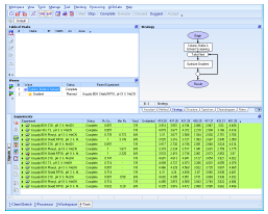
Mass Spec Data used for Automatic Peak Identification

Automatic peak identification was accurate except for isobaric compounds
Most of operator time was spent confirming peak identifications
Important to examine peak identifications for mixtures that are not well characterized.



Navigation from experimental design down to individual peak results was facile

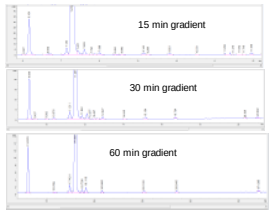
Screening output: Chose conditions to optimize



Primary criteria for screening: retention and peak shape
Resolution: It is possible that a column that gave lower resolution during screening will give superior resolution upon optimization..

Optimization Data

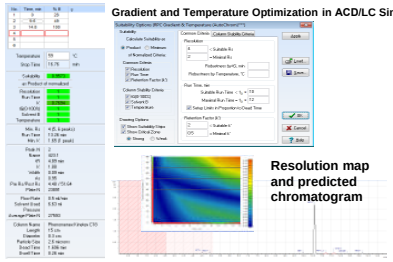
Input chromatograms for a gradient optimization



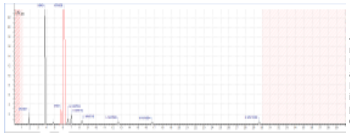
0.5 mL/min
A: 20 mM ammonium acetate pH=5.7
B: 10% buffer/90% acetonitrile
40°C
30-90% solvent/90 min
Poroshell PhenylHexyl column

Most optimizations performed with nine chromatograms: three different gradient slopes and three temperatures

Optimization

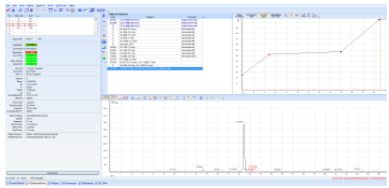


Single slope gradient from automatic optimization

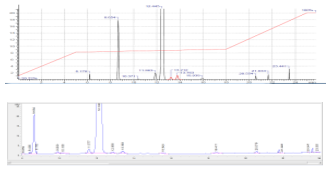


47-67% acetonitrile/35 min
Resolution of impurity after main peak: 1.5: However, for small impurity after main peak, need resolution of ≥3.

Manual adjustment of Gradient



Comparison of predicted and actual chromatograms



Measured retention times within 0.4 min of predicted

20-50% B/0-5 min, 50-53.7% B 5-18 min, 53.7-100% B 18-25 min
A: pH=5.7 ammonium acetate, B: 90:10 acetonitrile/buffer, 1 mL/min

Conclusions

Advantages of Autochrom versus manual collection
- No data conversions/importing
- Documentation of all phases of development
- Facile navigation of data sets
- Easy to combine data from multiple samples and signals to perform optimization
  - If one sample has all peaks and one signal has all peaks, this is not significant
  - If multiple samples and signals are to be used, manually identifying each component for each condition becomes daunting
- Peak identification is simplified

Software optimization of method conditions is a tremendous advantage

Disadvantages of Autochrom
- cost
- more complex
  - if only optimizing gradient with one signal and one sample, standalone version is just as good
  - If only need this ~once/year, then recommend working with an expert
- Peak identification is usually an advantage, except for isobaric compounds

Recommendations
- Software method optimization is invaluable tool for method development
- It is just a tool; not a replacement for planning and careful examination of results
- Software won't do your thinking for you; it makes the analyst more efficient and effective

If multiple complex method developments needed per year, automated method development like Autochrom would be invaluable.

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