

Practical Training Basic: LC Theory for Troubleshooting

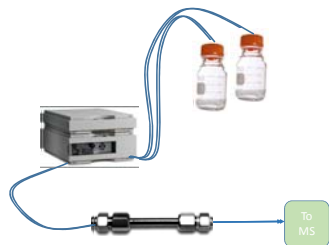
Autumn Breaud
Johns Hopkins University

LC Basics Part I:

Demystifying Liquid Chromatography Separations and Hardware

Introduction to Liquid Chromatography

- What is HPLC?
 - High performance liquid chromatography
- Principles of HPLC
 - Stationary phase
 - Mobile phase



Principles of HPLC

- Stationary phase + mobile phase + pump = separation



Principles of HPLC

• Stationary Phase

- Common column types
 - Non-polar
 - C18, C8, C4
 - Polar
 - Silica, amino, cyano
 - Ion Exchange
 - Cation and anion exchange
 - Size-exclusion
 - Phenyl/alkyl
 - Mixed mode
 - Combinations of above media

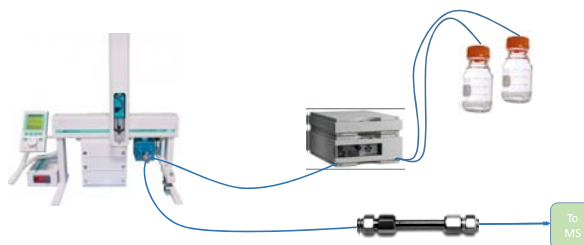
• Mobile Phase

- Aqueous: Water/Buffer
- Organic: Methanol/Acetonitrile
- Additives:
 - Acetic acid
 - Formic acid
 - Ammonium formate
 - Ammonium hydroxide
 - Ammonium acetate
 - TFA*

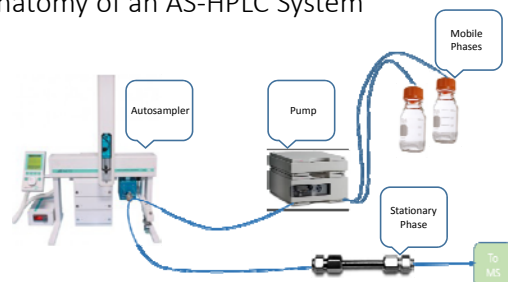
HPLC Hardware: The Missing Link(s)



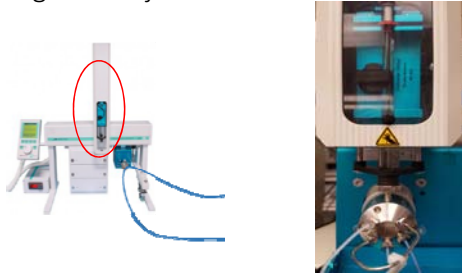
HPLC Hardware: The Missing Link: Autosampler



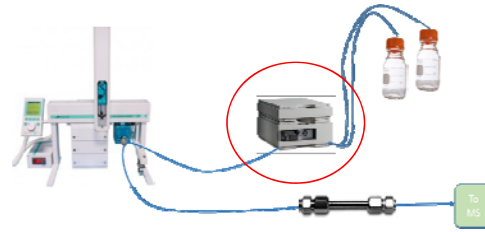
Anatomy of an AS-HPLC System



Anatomy of an AS-HPLC System: Autosampler Syringe and Injector Valve

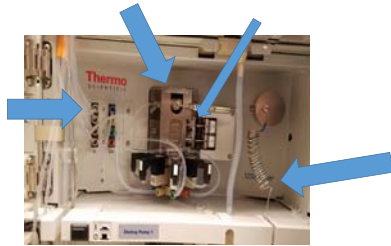


Anatomy of an AS-HPLC System: Pumps



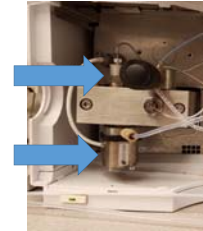
Anatomy of an AS-HPLC System: Common pump components

- Mobile phase inlet
- Mobile phase outlet
- Pump head
- Piston
- Check valve



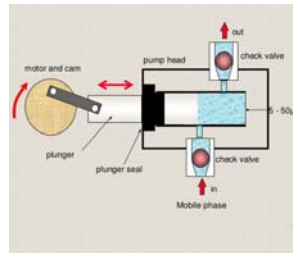
Anatomy of an AS-HPLC System: Common pump components

- Check valve
 - Inlet and outlet pictured



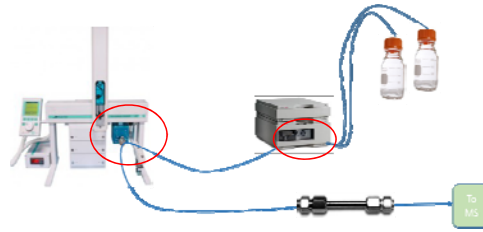
Anatomy of an AS-HPLC System: Check Valves

- What are they and why do I care?
 - Common cause of HPLC run failure
 - Stuck ball valve
 - Debris in valve chamber



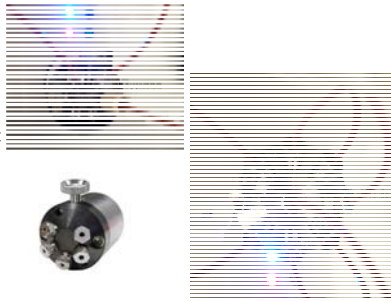
Slide Credit: M. Sagar Kishor Sawale

Anatomy of an AS-HPLC System: Valves



Anatomy of an AS-HPLC System: Valves

- Selection valves
- Bypass valves
- Injection valves
 - Common cause of HPLC run failure



Anatomy of an AS-HPLC System: Valves

- Stator face
- Rotor
- Rotor seal



Variations on a Theme: The Many Faces of Liquid Chromatography

- UHPLC
- Nanoflow/Microflow
- Multiplexing
- Multi-dimensional separations

Variations on a Theme: UHPLC

HPLC

- Particle size larger
 - 3-10 μm
- Longer run times
- Lower line pressure
 - 100-200 bar max



UHPLC

- Particle size smaller
 - 0.75- $<2\mu\text{m}$
- Shorter run times
- Higher line pressure
 - >500 bar max
- Improved resolution
- Lower sample and solvent volumes



Variations on a Theme: Nanoflow Chromatography

HPLC/UHPLC

- typical flow: 200-1000 $\mu\text{L}/\text{min}$
- column ID: 0.5-0.075mm

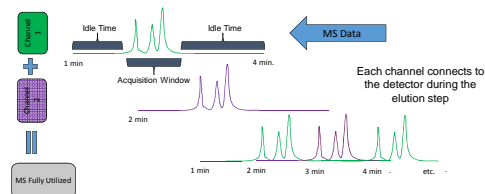


Nanoflow

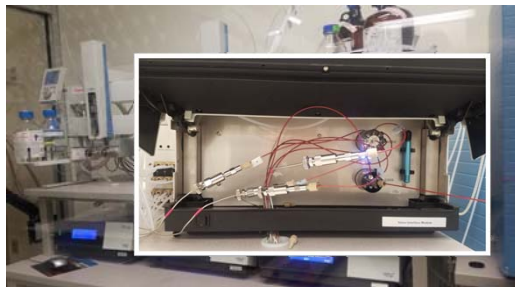
- typical flow <200 $\mu\text{L}/\text{min}$
- column ID: 2-4 mm



Variations on a Theme: Multiplexing



Variations on a Theme: Multiplexing



Variations on a Theme: Multi-dimensional Separations



LC Basics Part II:

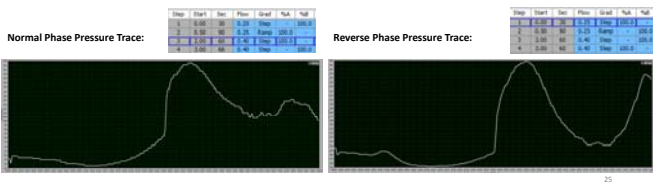
Troubleshooting and Staying Productive, or Up a Creek? Pick a Paddle

Troubleshooting LC Systems

- Diagnostic tools
 - Pressure trace analysis
 - Check valve failures
 - Empty sample/vial/well
 - Air in LC lines
 - Mobile phase composition errors
 - Loss of pressure analysis
 - Locate your clog
 - Lot check-in logs
 - Use them or lose it

Troubleshooting LC Systems: Pressure Trace Analysis

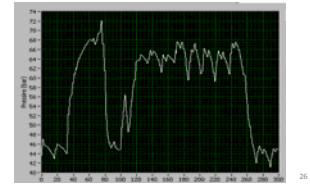
Step 1) Characterize a 'healthy' pressure trace:



Troubleshooting LC Systems: Pressure Trace Analysis

Step 2) Learn to 'read' aberrant pressure traces

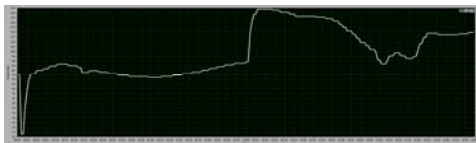
-Check valve failure



Troubleshooting LC Systems: Pressure Trace Analysis

Step 2) Learn to 'read' aberrant pressure traces

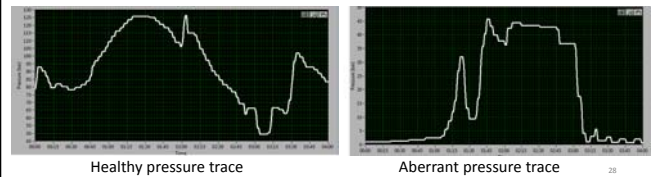
-Sample vial/well empty or AS does not transfer sample volume



Troubleshooting LC Systems: Pressure Trace Analysis

Step 2) Learn to 'read' aberrant pressure traces

-Empty mobile phase line/s

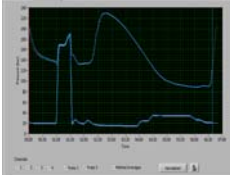


Troubleshooting LC Systems: Mobile Phase Composition Error

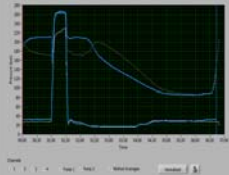
Step 2) Learn to 'read' aberrant pressure traces

-Mobile phase B topped off before run

Normal Pressure Trace



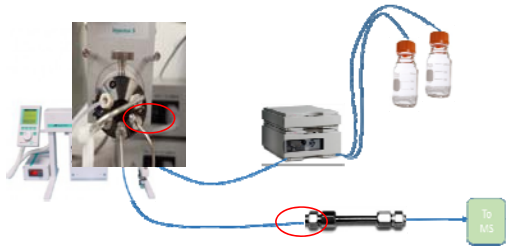
Problem Pressure Trace



29

Troubleshooting LC Systems: No Pressure

-Find that Clog

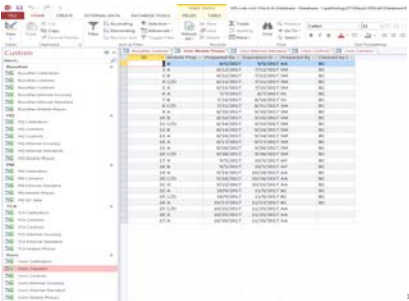


30

Troubleshooting LC Systems: Lot Change Tracking

Lot check-in logs:
Use them or lose it

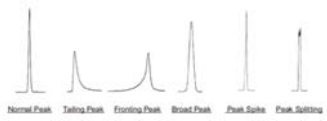
- Consumables:
- columns
- mobile phases
- internal standard solution lots



31

The Six Most Common LC Problems and How to Spot Them

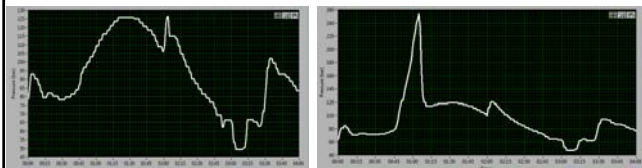
- Column aging and degradation
 - Peak tailing, fronting, broadening
 - Changes in retention time
 - Loss of signal



32

The Six Most Common LC Problems and How to Spot Them

- Clogging

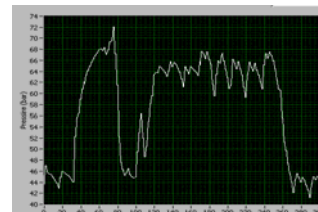


Healthy pressure trace

Aberrant pressure trace

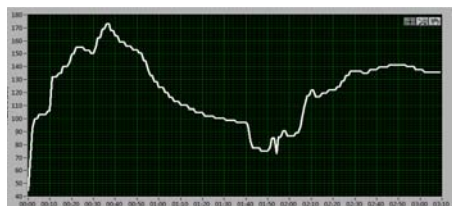
The Six Most Common LC Problems and How to Spot Them

- Check valve failure
 - Could be air in the line/s



The Six Most Common LC Problems and How to Spot Them

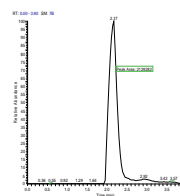
- Autosampler syringe failure



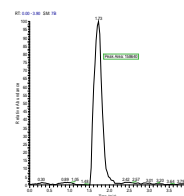
The Six Most Common LC Problems and How to Spot Them

- Incorrect mobile phase composition

Normal Chromatogram



Problem Chromatogram

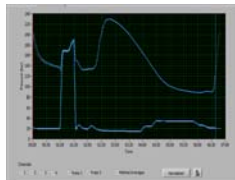


January 12, 2018

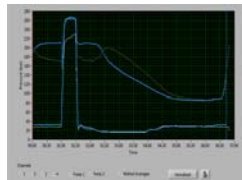
36

The Six Most Common LC Problems and How to Spot Them

Incorrect mobile phase composition



Healthy pressure trace



Aberrant pressure trace

January 12, 2018

37

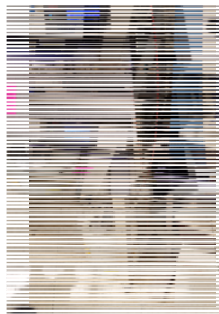
The Six Most Common LC Problems and How to Spot Them

- Best and most insidious problem:



The Six Most Common LC Problems and How to Spot Them

- Best and most insidious problem:



The Six Most Common LC Problems and How to Spot Them

- Best and most insidious problem:

- LC eluant on floor

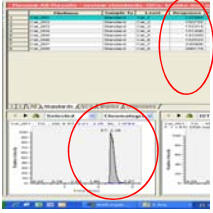
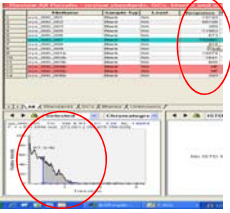


LC Basics Part III:

Case Studies in Separations Sleuthing

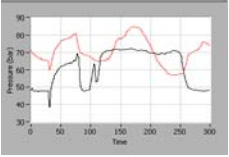
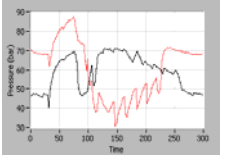
Case Studies in LC Troubleshooting

- Case Study 1:
 - The case of the vanishing busulfan peak

Case Studies in LC Troubleshooting

- Case Study 1:
 - The case of the vanishing busulfan peak
 - Pressure trace analysis

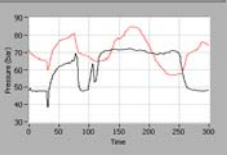



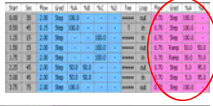
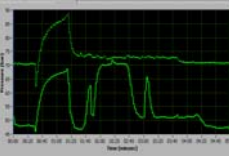
Healthy pressure trace

Aberrant pressure trace

Case Studies in LC Troubleshooting

- Case Study 1:
 - The case of the vanishing busulfan peak
 - Check valve replaced, new problem arises



Healthy pressure trace

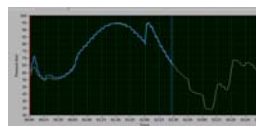
New aberrant pressure trace

Case Studies in LC Troubleshooting

- Case Study 1:
 - The case of the vanishing busulfan peak
 - Check valve failure
 - Gradient pressure profile still looks unusual
 - Mobile phase A (aqueous) as loaded in position of mobile phase B
 - Mobile phase A lot check-in procedure not completed, but bottle is labeled with new date
 - 'Marker test'
 - No elution of analyte
 - No pressure changes associated with solvent front or change in mobile phase surface tension from aqueous to organic

Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks



Healthy pressure trace



Aberrant pressure trace

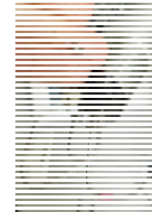
Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks



Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks



Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks



Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks
 - Flat pressure profile upon injection is aberrant and associated with missing internal standard signal
 - Change of AS syringe, needle, needle seal, injector rotor seal: still see intermittent signal loss



Case Studies in LC Troubleshooting

- Case Study 2:
 - The case of the intermittent missing antiretroviral peaks
 - Change of bungee cord: no more intermittent loss of signal
 - Pressure of injection was not high enough to overcome line pressure at moment of injection
 - Optimized needle penetration height in injector port



Speaker and
Presentation
Evaluation for Dr.
Autumn Breaud
Survey Monkey

<https://www.surveymonkey.com/r/7QMFZYJ>

Please let us know what training resources you need
chris.herold@msacl.org jastone@ucsd.edu