Review Article

A Review on HPLC-Trouble Shooting Guide

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ABSTRACT

This chapter gives an overview of HPLC system maintenance and summarizes strategies and guidelines for HPLC troubleshooting. It describes common maintenance procedures that can be performed by the user. Frequently encountered troubleshooting problems are classified into four categories (pressure, baseline, peak, and data). Common HPLC problems are caused by component malfunctions (pump, degasser, injector, detector, data system, column), and faulty preparation of the mobile phase or sample preparation. Each problem is described and symptoms and possible solutions are proposed. Most laboratories that work in a regulatory environment have an annual preventive maintenance program for their HPLC systems in which most of the wearable items are replaced. For a more detailed discussion of the subject, the reader is referred to textbooks,1–4 magazine columns,5 training software,6 manufacturers' operating and service manuals, and myriad web resources.

Keywords: HPLC, HPLC Trouble shooting, HPLC guide.

INTRODUCTION

HPLC

Although HPLC method has been improved by advances in column technology and instrumentation, problems still arise. The important segments of HPLC system at the same, whether we use a modular system or a more sophisticated unit.

Troubleshooting

Troubleshooting is a form of problem solving, often applied to repair failed products or processes. It is a logical, systematic search for the source of a problem so that it can be solved, and so the product or process can be made operational again. Troubleshooting is needed to develop and maintain complex systems where the symptoms of a problem can have many possible causes. Troubleshooting is used in many fields such as engineering, system administration, electronics, automotive repair, and diagnostic medicine. Troubleshooting requires identification of the malfunction(s) or symptoms within a system. Then, experience is commonly used to generate possible causes of the symptoms.

Troubleshooting Strategy and Processes[1]

Strategy

Any troubleshooting strategy involves five steps:

1. Identification of the problem
2. Awareness of the cause(s) of the problem
3. Isolation of the exact cause of the problem
4. Rectifying the problem if able
5. Returning the unit to routine use OR referring the problem to your maintenance manager.

Troubleshooting process

1. Gather the facts – not theories.
2. Check the simplest things first – it's easier.
3. Compare the performance obtained to the expected performance.
4. List possible causes.
5. Work through the possible causes in a step-by-step manner checking the outcome from any changes made.
6. As a last resort – get help from elsewhere, for example your instrument supplier help desk or your local technical support department.

General Problem Diagnostic and Troubleshooting Guide[2]

Topic: Baseline Symptom[6]

High baseline drift

1) Possible Cause: Detector lamp/optics temperature not stable

Solution: Allow the detector to warm up. Depending on the optical design, this may take 30 minutes to a few hours. See the detector's operating instructions for details.

2) Possible Cause: Mobile phase not homogeneous

Solution: After a day or more idle time, gently swirl the eluent bottles to homogenize eluents already in their reservoir.

3) Possible Cause: Column contamination

Solution: Flush the column with strong eluent. If possible, try to reverse the column (check with column specifications).

4) Possible Cause: Contaminated flow cell

Solution: Clean the flow cell (refer to the operating instructions for a recommended procedure). If necessary, replace the flow cell.

5) Possible Cause: New detector lamp

Solution: For a few hours, allow the lamp to reach a constant light intensity.

6) Possible Cause: Non-constant ambient conditions

Solution: Make sure that the temperature and the humidity in the laboratory are constant. Within Thermo Scientific Dionex Chromeleon, record temperature fluctuations with the help of detector temperature channels. Verify that the lamp and flow cell covers are in their proper position and that the front panel door is closed.

7) Possible Cause: Gradient operation (CAD)

Solution: Minimize gradient baseline effects by using appropriate mobile phase quality. Use shallow gradient methods whenever possible. Use inverse gradient.

8) Possible Cause: Insufficient equilibration time (ECD)

Solution: Wait until stable baseline (s) are achieved prior to performing analysis.

Topic: Baseline Symptom[4]

Non-periodic, high noise:

1) Possible Cause: Mobile-phase quality

Solution: Make sure to use HPLC-grade or better mobile phase quality, particularly for sub-2 µm particle columns and evaporative detectors (such as the CAD) use mobile phase with lowest particle content whenever possible.

2) Possible Cause: Too short response time (time constant) setting, increased noise Solution: Make sure to use suitable response time. Typically set a response time that is about 1/4 of the peak width at half-height of the narrowest peak. Follow the operating instructions for details.

3) Possible Cause: Too narrow optical bandwidth setting (UV detectors)

Solution: Use a low optical bandwidth setting for best optical resolution. Increase the bandwidth setting for best signal to noise. Please refer to the detector‘s operating instructions for details.

4) Possible Cause: Lamp is too old (optical detectors)

Solution: Replace the lamp. If after renewing the lamp the noise is high check that the lamp has been installed properly.

5) Possible Cause: Flow cell is contaminated, eluents are degraded.

Solution: Use fresh mobile phase, flush the flow cell according to the suggestions in the operating manual of the detector.

6) Possible Cause: Non-volatile mobile-phase (CAD)

Solution: Make sure to only use volatile buffers and additives with the CAD.

7) Possible Cause: Nebulizer (CAD)

Solution: Nebulizer may need to be cleaned. Remove column. Flush HPLC-CAD system with 80/20 water: methanol at 2.0 mL/min for several hours. Adjust to 0.2–0.4 mL/min overnight. Re-check noise level with fresh preparation of original mobile phase composition and flow-rate.

8) Possible Cause: Flooding of CAD

Solution: Perform self-test to ensure there are no errors present. Check to be sure all diagnostics are within specification.

9) Possible Cause: Nitrogen gas supply (for evaporative detectors such as CAD) Solution: Make sure to use 99% pure nitrogen, free from water vapour and particulates. Replace two-stage filter if necessary.

10) Possible Cause: Too narrow reference wavelength setting (DAD)

Solution: Choose wide bandwidth range of the reference outside the absorption range of the analytes. If possible, use a method without reference wavelength.

11) Possible Cause: Improper applied potential to working electrode (ECD)

Solution: Higher applied potential equals higher noise. Optimize the applied potential after performing an HDV (hydrodynamic voltamogram) for each analyte.

12) Possible Cause: Improper amperometric electrochemical cell assembly (ECD) Solution: Check the amperometric cell assembly. Be sure that there is a gasket present and that the target working electrode is in good physical condition. Replace gasket and/or electrode target if necessary.

13) Possible Cause: Contamination/ impurities (ECD)

Solution:

- Make sure to use low metal impurity buffer salts for the mobile phase whenever possible.
- Check the fluidics for metal contamination.
- Use lithium salts and /or use a microbicide additive when there is < 3% organic composition in the mobile phase.
- Replace mobile phase.

- Cell may require electrochemical treatment and/or cleaning with mobile phase. Aging cells may require replacement.

14) Possible Cause: Insufficient electrolyte (ECD)

Solution: Typical electrolyte concentrations of 25 mM - 100 mM are used in electrochemistry. For high applied potentials electrolyte concentration should be reduced to 15-25 mM.

15) Possible Cause: Too high temperature setting for Coulometric electrochemical cells (ECD)

Solution: Be sure to operate Coulometric cells at temperatures below 40°C.

Topic: Baseline Symptom

Periodic baseline fluctuation[4]

1) Possible Cause: Pressure fluctuations from pump

Solution: Record a pump pressure channel to identify temporary or permanent pressure ripple. A pump influence is indicated by baseline fluctuations synchronous with the pump cycle. Purge the pump, check general functionality (for instance with a diagnostics test), read pre-compression value from pump panel and compare against values from manual (Thermo Scientific Dionex pumps only). Check that appropriate mixing volume for applied eluents/flow rate is selected.

2) Possible Cause: Air in the pump fluidics

Solution:

- Degas eluents, best with on-line vacuum degassing or helium sparging.
- Purge the pump.
- Actively draw liquid through the pump, for instance with a syringe at the outlet of the pump.
- Temporarily replace the water with degassed organic solvent and purge the respective eluent line. Afterwards, switch back to degassed water.

3) Possible Cause: Insufficient mixing

Solution: Periodic, wave-like noise. Intensity of effect is related to the eluents, the type of detector, and the detector layout. Increase the mixing volume depending on the manufacturer's recommendation.

4) Possible Cause: Mobile phase degraded

Solution: Prepare fresh mobile phase, and flush the system with it. If required, back flush the column (increased backpressure).

5) Possible Cause: Improper grounding

Solution: Complete HPLC system should be plugged into the same circuit. Isolate the electrical circuit from strong current consumer devices or use an isolation transformer to filter current fluctuations.

6) Possible Cause: Wrong reference wavelength selected (DAD)

Solution: The sample must not absorb in the range of the reference wavelength. If possible, use a method without reference wavelength.

7) Possible Cause: Air inside electrochemical cell flow-path (ECD)

Solution: Place restrictive capillary tubing/inline filter on cell outlet to increase backpressure by 5 bar.

Topic: Baseline Symptom:

Slow column equilibration[3]

1) Possible Cause: Ion-pairing application

Solution: Normal. Ion pairing applications always require long initial equilibration times. Shorter alkyl-chain lengths of the ion-pairing agent allow for less equilibration than longer chains.

Topic: Baseline Symptom

Spikes[5]

1) Possible Cause: Air inside flow cell fluidics

Solution: Typically, bubbles appear randomly. Identify by,

- Observing similar spike heights at different wavelengths or
- Applying a slight backpressure on the flow cell (spikes disappear). Solution: Ensure that connections are tight. Degas mobile phase, use restriction capillary or backpressure regulator after flow cell (for instance 100 psi, check flow cell specifications).

2) Possible Cause: Column stored without caps

Solution: Flush column with strong mobile phase and make sure to store column properly, typically (RP-phases) in buffer-free, high organic liquid.

3) Possible Cause: Quick lamp intensity changes in the detection or reference wavelength.

Solution:

- Replace UV or Vis lamp.
- Check if the lamp is correctly inserted.

4) Possible Cause: Electrical interferences

Solution: Make sure to exclude the previous causes of spikes first. Spikes are typically not random, but for instance related to cyclic strong power consuming equipment. In addition, spikes can simply be caused by current fluctuations in the power grid.

Solution: Isolate the electrical circuit from strong current consumers or use an UPS (Uninterruptible Power Supply) to filter current fluctuations.

5) Possible Cause: Column temperature significantly above boiling point of the mobile phase

Solution: Use backpressure regulator after flow cell (for instance 100 psi, check flow cell specifications). Use post-column cooler (only available with selected column compartments, for instance the Thermo Scientific Dionex UltiMate 3000 TCC-3000RS). Consider switching off the flow cell heating.

6) Possible Cause: Drainage spiking (CAD)

Solution: Check for leaks at the waste bottle and drain/vent assembly. Check for the presence of internal tubing within drain line.

7) Possible Cause: Nebulizer (CAD)

Solution: Nebulizer may need to be cleaned. Remove column. Flush HPLC-CAD system with 80/20 water: methanol at 2.0 mL/min for several hours. Adjust to 0.2–0.4 mL/min overnight. Re-check noise level with fresh preparation of original mobile phase composition and flow-rate.

8) Possible Cause: Grounding (CAD)

Solution: Ensure that the CAD instrument is operating on the same circuit as the LC system. Ensure that the CAD instrument is plugged into a surge protector.

Topic: Changing Retention Times Symptom

Decreasing retention times[6]

1) Possible Cause: Degradation of stationary phase

Solution: Replace column. Check pH requirements of the column, typically pH 2–8 for silica-based RP columns.

2) Possible Cause: Insufficient equilibration

Solution: Increase equilibration times, at least 5–10 times the column volume.

3) Possible Cause: Column overload Solution: Reduce amount of sample. Increase column volume (use larger i.d.).

4) Possible Cause: Mobile phase composition

Solution:

- Check premixed mobile phase, make sure the liquid is homogeneous.
- Perform an OQ proportioning test.

a) High-pressure proportioning: Check for leaks or bubbles in aqueous eluent.

b) Low-pressure proportioning: Check/adjust proportioning valve.

c) Thermo Scientific Dionex UltiMate 3000 NCS-/NCP systems: Perform pressure sensor and viscosity calibration.

5) Possible Cause: Increasing flow rate

Solution: Check flow rate setting; perform OQ on flow rate precision.

6) Possible Cause: Stationary phase dewetting at low organic content

Solution: Use stationary phase compatible with low organic content (e.g., polar embedded phases). Re-wetting the column is facilitated by high organic content at increased pressure. Check column care manual for details.

Topic: Changing Retention Times Symptom

Elution window is narrower[3]

1) Possible Cause: Gradient delivery steeper than programmed

Solution: Check gradient settings, check solvents. Thermo Scientific Dionex UltiMate 3000 NCS/NCP systems: perform pressure sensor and viscosity calibration.

Topic: Changing Retention Times Symptom

Elution window is wider[3]

1) Possible Cause: Gradient delivery shallower than programmed

Solution: Check gradient settings, check solvents. Thermo Scientific Dionex UltiMate 3000 NCS/NCP systems: perform pressure sensor and viscosity calibration.

Topic: Changing Retention Times Symptom

Hydrophobic peaks in wash step (nano/cap)[3]

1) Possible Cause: Gradient delivery incorrect

Solution: Check gradient settings, check solvents.

Topic: Changing Retention Times Symptom

Increasing retention times[2]

1) Possible Cause: Mobile phase composition

Solution:

- Check premixed mobile phase, make sure the liquid is homogeneous.
- Perform an OQ proportioning test.

a) High-pressure proportioning: Check for leaks or bubbles in aqueous eluent.

b) Low-pressure proportioning: Check/adjust proportioning valve.

c) Thermo Scientific Dionex UltiMate 3000 NCS/NCP systems: Perform pressure sensor and viscosity calibration.

2) Possible Cause: Active sites on stationary phase

Solution: Use organic modifier base (such as TEA), increase buffer strength or use higher coverage column packing.

3) Possible Cause: Decreasing flow rate

Solution: Leaking capillary connections. Check flow rate settings.

4) Possible Cause: Pre-column dead volume (mainly nano/cap applications)

Solution: If peaks are late, but not broad there is a dead volume before the separation column. If the peaks are broad and late the dead volume is after the column

Topic: Changing Retention Times Symptom

Scattering retention times[5]

1) Possible Cause: Insufficient equilibration

Solution: Increase equilibration times, at least 5–10 times the column volume.

2) Possible Cause: Imprecise eluent proportioning Solution: Perform an OQ eluent proportioning test. Clean/replace pump check valves, or call service for adjusting/replacing proportioning valve.

3) Possible Cause: Insufficient buffer capacity

Solution: Increase buffer concentration > 20 mM. Work at pH within buffering range.

4) Possible Cause: Changing column temperature

Solution: Place column in column thermostat. Monitor oven temperature.

5) Possible Cause: No synchronization of injection with pump cycle (low-pressure proportioning)

Solution: Activate function in the software, for instance in the Thermo Scientific Dionex Chromeleon pump driver

Topic: Peaks Symptom

Broad peaks[6]

1) Possible Cause: Too large detector cell

Solution: Flow cell volume should not exceed 1/10 of the smallest peak volume. Use smaller volume flow cell (i.e., micro or semi-micro) with UHPLC or micro bore columns.

2) Possible Cause: Early peaks broader than later eluting ones

Solution: Too large extra column volume. Check capillary i.d. and length, sample loop size, flow cell, etc.

3) Possible Cause: Too long detector response time (time constant)

Solution: Select a response time less than 1/4 of the peak width at half-height of the narrowest peak. Use Thermo Scientific Dionex Chromeleon program wizard to optimize the response time (time constant) settings.

4) Possible Cause: High longitudinal dispersion

Solution: Isocratic separation: Retention time too long. Use gradient elution or stronger isocratic mobile phase; use less retaining stationary phase (e.g., C8 instead of C18). Linear velocity too slow: Check flow rate.

5) Possible Cause: Thermo Scientific Dionex Corona Detectors CAD compared to e.g., UV detection

Solution: CAD instrument will broaden peaks more than UV due to nebulization. Use Thermo Scientific Dionex Viper finger tight fitting system or Thermo Scientific Dionex nanoViper finger tight fitting system capillary connections for zero dead volume connections by design.

6) Possible Cause: Incorrect electrochemical cell configuration (ECD)

Solution: Check the fluidics of the System. Make sure to use the appropriate cell model post injection and in-sequence.

Topic: Peaks Symptom

Ghost peaks[6]

1) Possible Cause: Only some peaks broad: Late eluting peak from previous injection Solution: Extend run time. Increase elution strength of gradient (higher organic content). At the end of the run, flush column with strong eluent.

2) Possible Cause: Contamination (typically injector or column)

Solution: Flush sampler, replace parts prone to contamination (e.g., needle or needle seal). Flush column with strong eluent (also check column instructions; flush in reverse flow direction if possible).

3) Possible Cause: Contamination of eluents

Solution: Water quality: Run different amount of water over the column and measure enrichment time as a function of the volume. Replace water with HPLC-grade water. Contaminations may also result from bacterial growth in the degasser, eluent modifiers (e.g., degraded TFA) or improper liquid handling (e.g., amino acid mobile phase prepared without gloves).

4) Possible Cause: Interferences in sample

Solution: Use sample clean-up techniques such as SPE.

5) Possible Cause: Contaminated Nebulizer (CAD)

Solution: Nebulizer chamber may need to be cleaned. Remove column. Wash CAD instrument with 50/50 water/methanol at 2.0 mL/min for several hours. Adjust flow-rate to 0.2–0.4 mL/min overnight. Re-check noise level of original mobile phase composition.

6) Possible Cause: Microbial growth in a mobile-phase. Electrochemical cell electrode contamination (ECD)

Solution: Use fresh mobile phase with microbicide. Cell may require electrochemical treatment and/or cleaning with fresh mobile phase. Aging cells may require replacement.

Topic: Peaks Symptom

Intensity too low[6]

1) Possible Cause: Insufficient injection, detector insensitivity

Solution:

- Check the injection settings.
- Check sample concentration.
- Replace loop.
- Check detector settings and check flow cell.

Topic: Peaks Symptom

Missing hydrophilic peaks[6]

1) Possible Cause: Breakthrough of column

Solution: Check the solvents, gradient conditions, gradient delivery, and equilibration time. Perform viscosity measurement on Thermo Scientific Dionex UltiMate 3000 NCS/NCP systems. In preconcentration setups also check loading solvent and equilibration time of trap column.

Topic: Peaks Symptom

Missing hydrophobic peaks[6]

1) Possible Cause: Hydrophobic interaction of sample on vials, surfaces, etc.

Solution: Check sample age, sample preparation steps, check washing protocols.

Topic: Peaks Symptom

Negative peaks[6]

1) Possible Cause: Absorption/fluorescence of analytic is lower than mobile phase Solution:

- Change UV/fluorescence detection wavelength(s).
- Use mobile phase with less background absorption/fluorescence.
- Dissolve sample in mobile phase.

2) Possible Cause: Wrong polarization of analog output interface

Solution: Check cable polarity at analog output.

3) Possible Cause: Inappropriate reference wavelength setting (DAD)

Solution: The sample must not absorb in the range of the reference wavelength. If possible, use a method without reference wavelength.

4) Possible Cause: Drainage spiking (CAD)

Solution: Ensure that PEEK™ tubing is present within the drain/vent assembly. Check for gas leaks at the waste bottle cap and drain/vent assembly.

5) Possible Cause: The fluorescence of the substance is quenched by matrix or mobile phase (FLD).

Solution: 1. Evaluate changes to the composition of the mobile phase. 2. Consider using negative peaks for quantification.

6) Possible Cause: Possible reduction occurring at electrode (ECD)

Solution: Check applied potential(s) of the cell. Check the polarity of the affected channel(s) if working in reduction/oxidation mode.

Topic: Peaks Symptom

No peaks[6]

1) Possible Cause: Instrument failure, wrong elution conditions

Solution: Does the detector show typical baseline noise? If the detector output is just a flat line, the detector or the data transfer failed. Inject known test substance without column and check detector response.

2) Possible Cause: No injection, not enough sample volume

Solution: Ensure sample is drawn into sample loop. Check that injection has taken place (pressure drop at the beginning of run).

3) Possible Cause: High background current/noise (Charged Aerosol Detection (CAD) with Thermo Scientific Dionex Corona Detectors)

Solution: Check mobile phase quality. See baseline section.

4) Possible Cause: Sample too volatile (CAD)

Solution: Check response by flow injection analysis. Check vapours pressure of analyte(s).

5) Possible Cause: Settings of electrochemical detector (ECD)

Solution:

- Check to be sure that all electrochemical cells are powered "On".
- Check if potential is appropriate for analyte or channel (multi-channel cells). Optimize potential setting for each cell by performing HDV (hydrodynamic voltamogram) with the analyte.
- Check the gain range setting for each channel and set the appropriate level.

6) Possible Cause: Loose or incorrect electrochemical cell cable (ECD)

Solution: Check the integrity of the cell cable connections. Ensure that the correct cable is used to plug the cell into the detector.

7) Possible Cause: Potentiostat performance (ECD)

Solution: Check the potentiostat performance using appropriate simulator load(s).

8) Possible Cause: Contaminated reference electrode (ECD)

Solution: Flush the reference purge port on Model 5041/5041A and 5014B cells with fresh mobile phase. Possible Cause: Analyte auto-oxidation (ECD) Solution: Check for metal contamination that may cause analyte oxidation prior to detection at the electrochemical cell.

Topic: Peaks Symptom

Peak fronting[6]

1) Possible Cause: Blocked frit, particles on column head

Solution: Replace frit. If fronting returns quickly, try to locate source of particles (sample, eluents, pump mechanics, injection valve).

2) Possible Cause: Channels in column

Solution: Replace column. Check if application conditions are within the column specifications (e.g., pressure and pH range).

3) Possible Cause: Column overload

Solution: Reduce amount of sample. Increase column volume (use larger i.d.).

4) Possible Cause: Sample dissolved in strong eluent

Solution: Dissolve sample in starting mobile phase. Reduce sample solvent strength or injection volume.

Topic: Peaks Symptom

Peak tailing[6]

1) Possible Cause: Basic compounds interact with silanol groups

Solution: Use type B (high-purity) silica or shield phases (such as polar-embedded groups). Use competing base such as triethylamine (TEA). Alternatively (with type A silica columns), use polymeric columns. Use buffers of high ionic strength (displacement effects – NOT compatible with LC/MS).

2) Possible Cause: Insufficient buffer capacity

Solution: Increase buffer concentration.

3) Possible Cause: Chelation with trace metals in stationary phase

Solution: Add competing chelating agent (such as EDTA) to the mobile phase.

4) Possible Cause: Too large extra column volume

Solution: Use short capillary connections. Check inner diameter of connecting capillaries: 0.13 mm (0.005 in.) for UHPLC columns and 0.18 mm (0.007 in.) for conventional HPLC columns. The extracolumn volume should not exceed 1/10 of the smallest peak volume. Use Thermo Scientific Dionex Viper finger tight fitting system or Thermo Scientific Dionex nanoViper finger tight fitting system capillaries.

5) Possible Cause: Improper capillary connections

Solution: Check fittings for correct placement of ferrule. Use Thermo Scientific Dionex Viper finger tight fitting system or Thermo Scientific Dionex nanoViper finger tight fitting system capillaries.

6) Possible Cause: Column degradation

Solution: Replace column. With high temperatures: avoid combination with aggressive buffers (e.g., phosphate), reduce to uncritical temperature (e.g., 40 °C), and replace column type (e.g., hybrid column). With high pH: Use high-pH compatible column type.

7) Possible Cause: Column void (particularly at UHPLC pressures)

Solution: Replace column. Try to flush reversed column (outlet to waste). Preventive actions: Slowly increase the column flow to avoid pressure shocks on the column. Avoid aggressive pH (check column specifications). Routinely operate columns at less than 70–80% of the pressure specification.

Topic: Peaks Symptom

Split or double peaks[6]

1) Possible Cause: Contamination on column or guard inlet

Solution:

- Replace guard.
- Flush column with strong mobile phase, back flush to waste (if possible).
- Replace analytical column.

2) Possible Cause: Sample solvent too strong

Solution: Prepare/dilute sample in mobile phase.

3) Possible Cause: Coelution with unknown interference

Solution: Perform efficient sample clean-up. Adjust selectivity by changing the mobile phase or the column.

4) Possible Cause: Worn out rotor seal

Solution: Replace rotor seal. With extreme pH applications, check compatibility of the seal polymer.

5) Possible Cause: Packing integrity loss (UHPLC applications)

Solution: Replace column. Check pressure rating of the column and work at maximum 70–80% of the pressure spec. slowly increase pressure on columns to avoid pressure shocks. Possible Cause: Temperature mismatch Solution: Mainly occurs with column i.d.'s > 3 mm in combination with high column temperatures. Use an eluent pre-heater to ensure eluent temperature does not result in temperature gradient over column.

6) Possible Cause: Improper capillary connections

Solution: Check fittings for correct placement of ferrule. Use Thermo Scientific Dionex Viper finger tight fitting

system or Thermo Scientific Dionex nanoViper finger tight fitting system capillaries, which provide zero dead volume connections by design. Possible Cause: Air inside electrochemical cell flow-path (ECD) Solution: Increase backpressure by approximately 5 bar by placing a restrictive capillary tubing/inline filter on cell outlet.

Topic: Peaks Symptom

Too low signal-to-noise[6]

1) Possible Cause: Non-ideal fluorescence detector settings

Solution: Scan for best excitation and emission wavelengths, optimize the gain of the photomultiplier, use high-quality mobile phase only, set suitable response time.

2) Possible Cause: Non-ideal UV detector settings

Solution: Scan for best absorption wavelength(s), set suitable response time and optimize slit widths and bandwidths according to the operating manual.

Topic: Peak Area Precision Symptom

Analyte transfer from previous injection[6]

1) Possible Cause: Carry over (from needle, sample loop, needle seat)

Solution: Extend auto sampler wash options to reduce carry over. Clean needle, needle seat, and rotor seal.

2) Possible Cause: Column memory effect

Solution: Run blank sample (no injection) after sample. If a peak is detected, then a column memory effect is present. Flush column with strong eluting eluent. Reverse column (if allowed) and flush column again. Replace column.

Topic: Peak Area Precision Symptom

Caused by instrument or sample[6]

1) Possible Cause: Sample or sampler problem

Solution: Perform multiple injections to differentiate between sampler and sample related issues:

- Variations of the sum of the peak areas: Injector.
- Only some peak areas vary: Sample not stable. To verify: Inject known stable mixture: peak areas should not vary.
- If peak areas of some peaks still vary, check system pressure stability and short term flow stability.

Topic: Peak Area Precision Symptom:

Dominant peak shift/cluster shift (ECD Array)[6]

1) Possible Cause: Change in mobile-phase composition/age of mobile-phase Solution: Replace mobile phase with fresh supply.

2) Possible Cause: Older Electrochemical Array cell pack

Solution: Replace array cell pack.

3) Possible Cause: Electrode contamination

Solution: Electrodes within the cell may require electrochemical treatment and/or cleaning with mobile phase. Aging cells may require replacement.

Topic: Peak Area Precision Symptom

Fluctuating baseline[6]

1) Possible Cause: Irreproducible integration caused by pump pulsation, mixing ripple, etc.

Solution: Please refer to the baseline table.

Topic: Peak Area Precision Symptom:

Inappropriate detector settings[6]

1) Possible Cause: Wrong detection wavelength(s)

Solution: Measuring in a UV/fluorescence spectrum flank can compromise the precision. Choose a detection wavelength or an excitation/emission wavelength pair near the apex of the spectrum/spectra. If spectra of analytes are very different, a wavelength switch might be required.

2) Possible Cause: Response time too short, high noise, imprecise integration at trace level

Solution: Make sure to use suitable response time (or time constant) settings. Typically set a response time that is about 1/4 of the peak width at half-height of the narrowest peak. Follow the operating instructions for details.

3) Possible Cause: Incorrect nebulizer temperature (Thermo Scientific Dionex Corona Detectors ultra (RS)

Solution: Check nebulizer temperature setting. If using large amounts of THF or halogenated solvents in mobile phase, set temperature to 30 °C. If analyte is semi-volatile, it may be necessary to turn off the nebulizer heater in order to recover response.

4) Possible Cause: Not enough data points

Solution: Set the data collection rate at least to 20-30 data points for reproducible peak integration.

Topic: Peak Area Precision Symptom

Injection volume variation[6]

1) Possible Cause: Auto sampler draws air from the vial

Solution: Check the sample filling height and the sampling height of the injector needle.

2) Possible Cause: Sample degradation

Solution: Use appropriate storage conditions, e.g., thermostatted auto sampler.

3) Possible Cause: Air in the auto sampler fluidics

Solution: Flush out auto sampler fluidics following the steps laid out in the respective operating instructions.

4) Possible Cause: The auto sampler, the injection valve, and/or the syringe valve are not tight

Solution: Check seals and tighten fittings involved in the injection process of the sampler.

5) Possible Cause: Injector needle clogged or needle tip deformed

Solution: Replace needle. Remove air from auto sampler fluidics.

6) Possible Cause: Auto sampler draw speed too high, sample with high gas content Solution: Reduce the draw speed to take at least 2–3 seconds. Program a delay time after the drawing of sample (e.g., 3 seconds). Degas sample and/or decrease draw speed.

7) Possible Cause: Leaking injector seal, bubble in syringe

Solution: Check injector seals, purge syringe.

Topic: Peak Area Precision Symptom

Peak Integration Settings[6]

1) Possible Cause: Positions of integration delimiters vary

Solution: Check the software integration settings. For instance, with the support of the Thermo Scientific Dionex Chromeleon help. Best, facilitate the Chromeleon 7 Cobra algorithm and let it identify the ideal settings for you. Avoid automatic data rate settings and use a fixed data rate.

Topic: Pressure Symptom:

Fluctuating pressure[6]

1) Possible Cause: Air trapped in pump

Solution:

- Degas eluents, best with on-line vacuum degassing or helium sparging.
- Purge the pump against a gentle backpressure.
- Temporarily replace the water with degassed organic solvent and purge the respective eluent line. Afterwards, switch back to degassed water.

2) Possible Cause: Inlet or outlet check valves of the pumps are dirty

Solution: Take out check valve cartridges, place them in a container with a suitable liquid (e.g., methanol) and clean them in an ultrasonic bath.

3) Possible Cause: Failing pump check valves or piston seals

Solution: High-pressure proportioning: Identify the failing pump block by relating the pressure drops to the pump cycle. Change the % B and observe how the frequency of pressure drops changes. Use Thermo Scientific Dionex Chromeleon pump diagnostics to identify the failing part.

4) Possible Cause: Blocked solvent line frit

Solution: Replace solvent line frit.

Topic: Pressure Symptom

Too high/increasing pressure[6]

1) Possible Cause: Buffer precipitation

Solution: 1. Check buffer compatibility with max organic content (in beaker). 2. Flush out system and column with low organic content. 3. Systematically investigate fluidics to locate the blocked part (from detector to pump).

2) Possible Cause: Bent capillaries

Solution: Systematically investigate fluidics to locate the blocked part (from detector to pump).

3) Possible Cause: Particles on mixer, frit, guard, or column

Solution: Use pre-filtered mobile phases suitable for the used column particle size (for instance 0.1-0.2 µm for sub-2 µm particles, 0.45 µm for 3–5 µm particles). Filter samples accordingly. Check sealing mechanic parts (piston seal, rotor seal).

4) Possible Cause: Too high flow or too low column temperature setting

Solution: Correct setting.

5) Possible Cause: Column aging/blocking Solution: Gradual pressure increase is normal over the lifetime of a column. Unusual pressure increase: Check connection tubings, temperature control, replace column.

6) Possible Cause: Pressure changes with the course of a gradient

Solution: Normal. The system pressure is dependent on the viscosity of the mobile phase composition in the system fluidics.

7) Possible Cause: MS needle blocking

Solution: Check MS needle, check connection tubings.

8) Possible Cause: Blocked/clogged liquid inlet or nebulizer (CAD)

Solution: Check backpressure of the CAD instrument. Backpressure should be under 10 bar (145 psi).

9) Possible Cause: Blocking or clogging in electrochemical detector fluidics (ECD) Solution:

- Check inline PEEK™/graphite filter(s) and replace the elements as necessary.
- Blocked or clogged cell may require electrochemical treatment. Reverse flow of mobile phase through cell. Flush cell with alternating miscible organic and aqueous mobile phase. Aging cells may require replacement.

Topic: Pressure Symptom

Too low pressure[6]

1) Possible Cause: Leaking capillary connections

Solution:

- Check fittings for tightness, particularly before the column. PEEK™ capillaries with finger tight fittings may slip out of the ideal position.
- Use Thermo Scientific Dionex Viper fingertight fitting system or Thermo Scientific Dionex nanoViper fingertight fitting system capillary connections for zero dead volume connections by design.

2) Possible Cause: Too low flow or too high column temperature setting Solution: Correct setting.

Topic: Pressure Symptom:

Trap column pressure too high (constantly)[6]

1) Possible Cause: Trap column clogging

Solution: Cartridge trap column: reconnect inlet/outlet tubings and reverse column, replace cartridge trap column. Nanotrap columns: 1. Flush with high organic solvent overnight. 2. Replace nanotrap column.

CONCLUSION

Developing HPLC troubleshooting skills often takes many years of operating experience and a working understanding of the principles of the instrument as well as considerable patience to eliminate all the typical causative factors.

HPLC column problems are evident as

1) High pressure (prevention better than the cure)
   a) Undesirable peak shape
   b) Changes in retention/selectivity
2) Often these problems are not associated with the column and may be caused by instrument and chemistry issues.
   a) pH of mobile Phase
   b) Instrument Connections
   c) Detector Settings
   d) Metal Contamination
3) Start With the Correct Questions
   a) Find the Answers
   b) The Answers will Lead to Solutions

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