

Troubleshooting and How to Take Care of Your Column and HPLC System

HPLC Troubleshooting

- n Pressure: too much or too little
- n Leaks: pump, autosampler, detector
- n Reproducibility: pump, autosampler

- n Column Care: Flushing and equilibration

Pump Troubleshooting

- n No pressure, or fluctuating pressure
 - n Pump may not be completely full of liquid – check solvent inlet line
 - n Air in check valve – always degas mobile phase!
 - n “Stuck” (κολλημένη) check valve – the pump may have been idle (αδρανής) for too long and solvent has dried inside the check valve.

Poor quality solvent: may contain resins that coat the ball (σφαίρα) inside the check valve, and that film won't let the ball seat properly

Pump Troubleshooting

n High Pressure

n Outlet frit (φρίτα, πορώδες φίλτρο) may be blocked with particles from mobile phase or seal (φλάντζα) material

n Leaks (διαρροές)

n Damage to seal and/or plunger (έμβολο) due to several factors

n Misaligned (μη ευθυγραμμισμένο) plunger

n Solvent incompatibility with seal material

n Salt crystal buildup from buffers – use a rinse kit!

Pump Troubleshooting

- n Retention Time Reproducibility
 - n For a dual piston pump, only one side may be filled with liquid – check solvent inlet lines
 - n Temperature change (may not be the pump's fault)
 - n A 1° shift in temperature can result in a 1-2% shift in retention time
 - n Avoid drafty (μη θερμενόμενες) locations in the lab
 - n Use a column oven when possible

Autosampler Troubleshooting

n High Pressure

- n Particulates from mobile phase, sample, pump may be trapped (παγιδευτούν) in the inlet tubing or valve
 - n Filter mobile phase AND sample when possible

n Leaks

- n Fittings may be loose on the valve
 - n Tighten (σφιξτε) fittings (προσαρμογές) properly and don't exceed the pressure limit of the autosampler

Autosampler Troubleshooting

n Area % Reproducibility

- n Always degas rinse phase (απαέρωσε τη φάση έκπλυσης) , and use some volume of liquid for rinsing to keep all flow paths in the valves full of liquid
- n Make sure the needle stroke (κτύπημα της βελόνας) is deep enough to draw sample from the vial
- n Check for leaks on the valve fittings, and the connection to the column inlet

Detector Troubleshooting

n Spiky Baseline (γραμμή βάσης με οδοντώσεις)

- n Air bubble in flow cell – degas mobile phase!

- n Put some restriction (συμπιεστή) on the cell outlet, but not too much! Tubing with 0.005” i.d. is fine.

n Leaks

- n Cracked flow cell (ραγισμένη κυψελίδα)

- n Don't exceed the pressure limit of the cell

- n Poor tubing connections

- n Use the proper fittings and tighten appropriately

Column Care

- n Follow MER's (κατασκευαστής) recommendations for solvent compatibility (συμβατότητα), flow rate, and pressure limits
- n Filter samples when possible
 - n Particulates will build up on the inlet frit over time
- n Use care when reversing (αντιστροφή) column flow
 - n Connect the outlet to waste, NOT inline with the detector to prevent further contamination
- n Store columns in recommended solvents

Troubleshooting Summary

- n Throw away bad parts and columns.
- n Leaks do not fix themselves.
- n If it doesn't pass, you must degas.