

TROUBLESHOOTING GUIDE – HPLC

Before starting any troubleshooting, whether it is related to instruments or columns, it is essential that safe laboratory practices be observed. The chemical and physical properties of any solvents used should be known and the material safety data sheet (MSDS) for these solvents should be readily available.

All electrically powered instruments should be powered down and unplugged from the main supply before the removal of their covers, etc...

Wear eye protection when troubleshooting the detector (with the cover removed) as ultraviolet light is emitted during UV and fluorescence detector operation and this will damage the eye's cornea irreversibly.

There are many areas in a HPLC instrument that give rise to system and chromatographic problems. This guide will deal with each one in the following sections:

- visual inspection
- pressure
- baseline irregularities
- changes in chromatography
- qualitative results
- quantitative results

I- Visual inspection

When a problem occurs, it is advisable to perform a quick visual check of the instrument and column. This will pick up leaks, loose or disconnected tubing, changes in instrument settings, etc...

II- Pressure

System pressure is affected by a number of variables including the viscosity of the solvent used, column variables, flow rate and temperature. It is important to have a reference point when high or low pressures to the norm. This reference point should be the pressure generated in the system when everything is functioning correctly.

Pressure problems fall into one of three categories: high, low or fluctuating pressure. They can occur suddenly or be a gradual process. Sudden pressure rises tend to be due to particles from the sample, blocked or damaged tubing or column packed bed collapse. Gradual pressure rises can also be due to particles in the sample, but they can also arise from particles generated in the instrument, for example, debris from vial septa or degrading seals.

Before releasing any high pressure build-up in a system, be aware that the solvent may form an aerosol or spray when loosening connections. Eye protection should be worn and ideally the connection to be positioned above and adsorbent material to soak up all released solvents.

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Rachel Sandford



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