Troubleshooting Problems With Poor HPLC Results Before Examining the Column - Tips & Suggestions

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When chromatographic results are poor, do not immediately assume the column is at fault.

Before spending time troubleshooting the HPLC column, rule out other, easier-to-fix possibilities first. Issues with peak shape, retention, and similar problems are often caused by components of the HPLC system. Consider the following real-world example from our laboratory.

Before suspecting the column, check the following:

• **Mobile phase:** Are A and B lines connected correctly? Has flow rate calibration been verified for each line? Is the proportioning valve functioning accurately? When was the mobile phase prepared, and does it need replacing?

• **Method:** Is the mobile phase or gradient appropriate for your compounds? Use ANP gradients for polar compounds and RP for less polar analytes. Are you using the correct detection wavelength? Review method settings to ensure nothing was changed by another user.

• **Sample preparation:** Are analyte concentrations suitable? Avoid column or detector overload by diluting if necessary. Have samples been filtered to remove particulates? Have recovery studies confirmed analytes are present in the final sample?

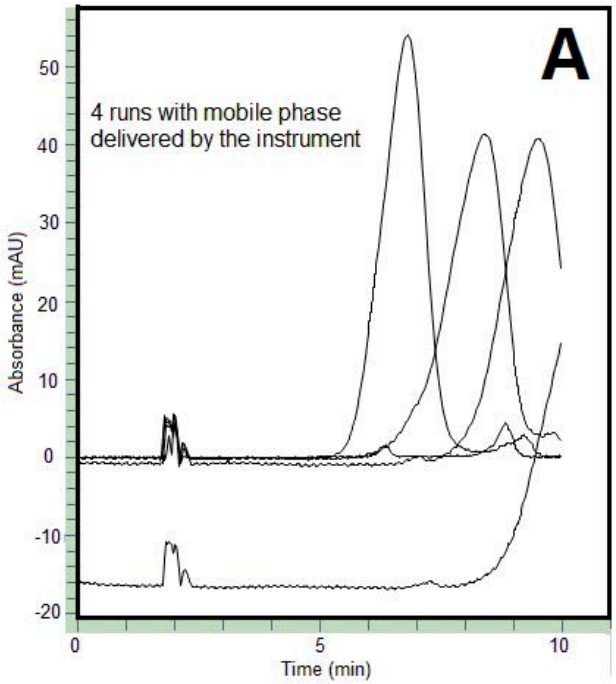
• **Injector:** Is the needle reaching the liquid sample? Is the syringe aspirating the correct volume?

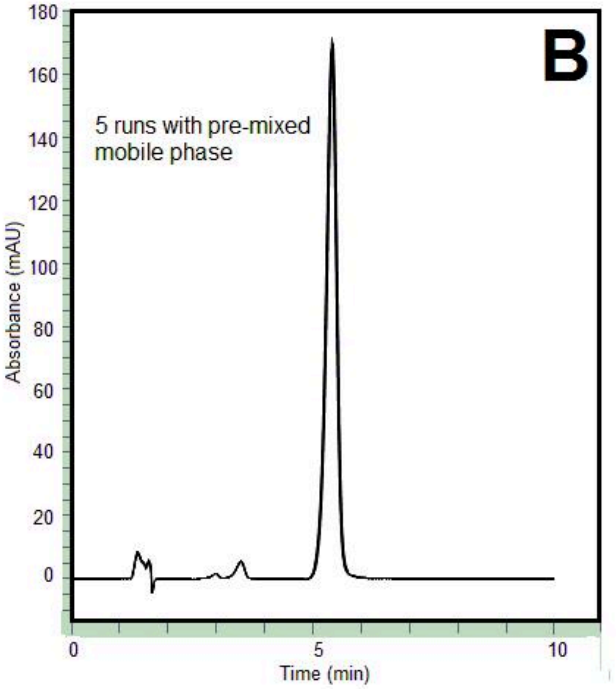
This is not a comprehensive troubleshooting list, but it highlights common system-related issues. HPLC analysis involves many factors, so do not start by blaming the column.

Case Study: We were running an HPLC method for ethylbenzene (0.1 mg/mL concentration) using a Cogent UDA™ column and observed problems with the chromatograms. The analyte peak was retained longer and longer after each injection, and the peak became broader. Was this due to column damage? Knowing the columns are very stable, we investigated other likely causes first.

We were using a 0.5 mL/min isocratic mobile phase from two solvent reservoirs (70% solvent A, 30% solvent B). Suspecting an issue with one of the solvent lines, we prepared a premixed mobile phase and used only one reservoir (solvent A). Under these conditions, retention time precision improved significantly and efficiency was high. This showed the column was fine and the problem was with the solvent B line, possibly at the mixing stage where the proportioning valve was not delivering the correct amount of solvent.

Another issue was a smaller peak that appeared inconsistently. Investigation revealed a problem with the injector. We were using a 1 µL injection volume; increasing it to 5 µL made the peak consistently present.





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MicroSolv Technology Corporation
9158 Industrial Blvd. NE, Leland, NC 28451
Tel: (732) 380-8900
Fax: (910) 769-9435
Email: customers@mtc-usa.com
Website: www.mtc-usa.com