

## Can Low Quantitative Results in HPLC be Due to Autosampler Vials or Caps - HPLC Primer

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**Issue:** I am running HPLC methods and observe low analyte quantitation.

### Other observations :

- The problem appears to be random in nature
- Three different autosamplers are involved
- Mobile phases and samples are the same on other instruments that do not exhibit this behavior
- Replacing the needle does not change the low result
- Use of partial or pre-slit septa eliminates the problem
- The autosampler loop hasn't been modified as compared to the other instruments
- No obvious coring is present in the syringe or autosampler vial
- Samples are filtered or not treated differently than on the other instruments

**Solutions:** There are several possible explanations for the low result. If you determine that the cause is due to an inaccurate volume of the aspirated sample in to the autosampler, you can then further trace the problem to one of two possibilities.

**First**, there could be a leak (*air gap*) or debris randomly orienting itself in such a manner as to inhibit full aspiration. Try inspecting both sides of the vial cap septa and see if any material is missing. If so, this debris could be the culprit. Sometimes this is not easy to see since the missing material could be inside the pierced septa.

**Second**, check your method's aspiration speed to ensure that it is not too high. Viscous samples require a lower draw speed or else a partial fill may occur.

Aside from the needle itself, It is possible there is a leak elsewhere in the injection system (*from the needle connection all the way through to the switching valve and/or injection valve*) . A leak in the switching valve is less random however. A leak between the autosampler and injector port may need considerable servicing to correct and often requires complete autosampler replacement. Try checking all the connections for a leak. You can also visually observe the needle when it is in the process of injection. Check for misalignment of the needle with respect to the vial.

If use of solid septa appears to be the differentiator and changing to partial or pre-slit septa resolves the problem, it may indicate either a pressure differential function (*i.e. vapor lock, partial vapor lock, or instantaneous pressurization during penetration*) condition or alignment issue. Again, partial or no-fill pull may not necessarily indicate an over filled autosampler vial or coring but this doesn't

eliminate either possibility. The angle of injection as well as depth can lead to random sampling errors. Please make sure the offending vial had the same area of septa penetrated and that the needle did not drift or deflect.

[RSA Vials, Caps & Insert Ordering Information](#)

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