

## Not Seeing an Expected Peak in an HPLC Assay - Tips & Suggestions

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### Troubleshooting: No Peak Detected

If you do not see a peak during analysis, follow these steps to investigate possible reasons:

#### 1. Check Column Connection

- Detach the column and replace it with a column union.
- Inject the solution again.
  - **If a peak appears:** The issue may be with your column (e.g., poor retention or elution).
  - **If no peak appears:** Continue to the next steps.

#### 2. Verify Sample Preparation

- Ensure the analyte is properly extracted and present in the sample.
- Confirm the sample level is sufficient for the autosampler syringe.
  - If the level is too low, the needle may aspirate air instead of the sample.

#### 3. Inspect Detector Performance

- A malfunctioning detector can prevent peak detection. Check for proper operation.

#### 4. Confirm Flow Path

- On systems with multiple column options, verify that flow is directed to your column.
- If there is **no pressure** with the column connected, flow may not be reaching it.

