Negative Baselines Observed in HPLC Runs at 214 nm - Tips & Suggestions

Why Does My Baseline Go Negative When Switching Gradients?

Question:
When I run gradients on my HPLC and switch from one gradient to another, my baseline appears negative. If I auto-zero, it becomes positive for the remainder of the run, but when I start again, it goes negative. What can I do?

Explanation

Negative baselines during gradient runs are not uncommon. If your method uses components that absorb UV—even moderately—at the detection wavelength, it is challenging to perfectly balance absorbance between channels.

- Acetic acid is particularly problematic in this regard.
- The lower the wavelength, the harder it is to manage.
- For example, achieving a smooth gradient with peptides at 214 nm using TFA illustrates this difficult

The only suggestion we can have for you is to start with fresh mobile phase and to make sure that your reagents and solvents are as pure as you can afford. Sometimes columns will retain these impurities from the solvents and reagents and will release during a run.

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