Negative Baselines in HPLC at 214nm – Tips & Suggestions

When I run gradients on my HPLC and switch from one gradient to another, my baseline shows as "negative". If I auto zero, it becomes positive for the remainder of the run but when I start again, it is negative. What can you suggest?

Negative baselines in gradients are not that unusual. If the method is using even moderately UV absorbing components at the wavelength of interest, it is very difficult to exactly balance the absorbance signals of both channels. Acetic acid is very bad in this respect. The lower the wavelength, the more difficult it is to manage. Also, getting a smooth gradient with peptides at 214nm and TFA is an excellent example of the challenge at hand.

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.

If you observe this, I suggest you also use a fresh column.

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