

How to eliminate retention time drift in LCMS with Aqueous Normal Phase ANP methods – Tips & Suggestions

A significant run to run Retention Time drift could be possible in some ANP methods: A case study to eliminate it.

Up to 1 minute for 10 repeated runs, using the Cogent Diamond Hydride™ HPLC column, showed Retention Time drift. After conditioning the column with 90% IPA and 10x 1% phosphoric acid injections as suggested for our specific method, we were able to see our compounds of interest. However, we could not get reproducible retention time in repeated sample runs with our sample matrix. I have tried extending the aqueous wash to 20min, still no help. Do you think this is due to our LC or column problems?

I don't think this is a Column issue. The mobile phase is similar to one I used in the vitamin separation (*Ammonium Acetate in the B solvent, acid in the A solvent*). I did notice retention drift issues if the column was not thoroughly equilibrated between runs. Please try having the gradient not go all the way to 100% A. There will always be some ammonium acetate in the mobile phase.

Ammonium Acetate takes some time to load and remove from the column. If there is always some Ammonium Acetate present, it will not have to be completely removed and then re-adsorbed on the surface for the next run.



Also, I think the column may need 30 minutes more conditioning with solvent A (50% DI water/50% isopropanol), before use for this method.

Customer METHOD CONDITIONS:

Mass Spec: Negative ion

Flow rate: at 0.6 ml/minute

Column Temperature: 40° C

Solvent A: 50% water / 50% isopropyl alcohol / 0.025% acetic acid and 5 uM EDTA

Solvent B: 10% water / 90% acetonitrile with 5 mM Ammonium Acetic with 5 uM EDTA adjusted to pH 7.0 using NH3

Injection Volume: 2 ul

Sample dissolved in 50% Acetonitrile/ 50% Water 0.2% Ammonia

GRADIENT

T0 99%B

T1 99%B

T15 20%B

T15.1 0B

T29 0B

Stop: 29 minutes

Post Time: 7 minutes

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