

Why am I observing broad split peaks in Aqueous Normal Phase ANP when using acetonitrile and methanol diluents – FAQ

Question: I am analyzing pyrazinoic acid (see structure below) by **Aqueous Normal Phase ANP HPLC** using the **Cogent Diamond Hydride™** column. I observe two broad, split peaks for the **analyte**. I am using an acetonitrile / DI water / formic acid based mobile phase and my diluent is 50:50 methanol / acetonitrile. Retention is also low. **What can I do to improve peak shape and / or retention?**

Answer: The absence of water in your diluent may be contributing to the split broad peak shape. This has often been observed when using non-aqueous containing diluents with an aqueous/organic ANP mobile phase. Try a diluent of 50:50:0.1 acetonitrile / DI water / formic acid. If that does not solve the problem, try an ammonium acetate-based mobile phase and diluent (10mM).

You will probably be using negative ion mode LCMS in that case. With the carboxyl group ionized, you will probably be looking for the $[M-H]^-$ ion. You can expect stronger retention with the carboxyl group ionized.

