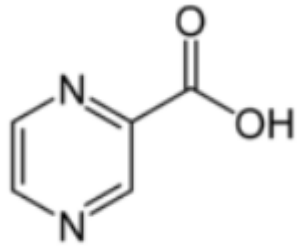
Why am I observing broad split peaks in Aqueous Normal Phase ANP when using acetonitrile and methanol diluents – FAQ

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.



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