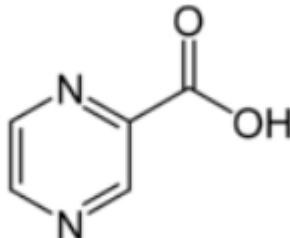


## Eliminate broad split peaks in Aqueous Normal Phase ANP methods and a methanol diluent- Tips & Suggestions

**Case Study:** When analyzing Pyrazinoic Acid with an Aqueous Normal Phase HPLC ANP method and 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?

**Suggestion:** 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 in that case. With the carboxyl group ionized, you will probably be looking for the [M-H]<sup>-</sup> ion. You can expect stronger retention with the carboxyl group ionized.



Pyrazinoic Acid