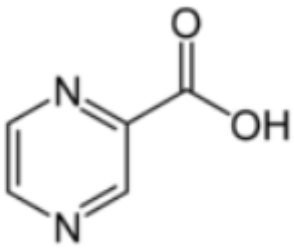
Eliminate broad split peaks in Aqueous Normal Phase ANP methods and a methanol diluent- Tips & Suggestions

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]- ion. You can expect stronger retention with the carboxyl group ionized.



Pyrazinoic Acid

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MicroSolv Technology Corporation
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tel. (732) 380-8900, fax (910) 769-9435
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