Lower Retention is Observed When Functional Group in a Compound Should be Ionized - Troubleshooting Tips

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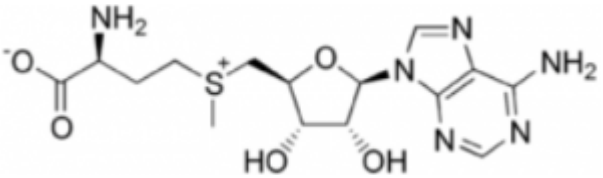
In analyzing S-adenosyl methionine *(SAMe)* with the Cogent Diamond Hydride™ column with an Aqueous Normal Phase ANP HPLC method, retention time may be reduced under the following conditions:

First gradient with a formic acid additive and then used an ammonium acetate additive. With ammonium acetate, lower retention is observed than with formic acid using otherwise identical method conditions.

One might be expecting greater retention with ammonium acetate since the carboxyl group would be ionized at this pH but not with formic acid. Also expected the compound would be more polar if it were ionized and therefore would retain longer. What happened?



Suggestion: The compound also has a permanent positive charge on the sulfur functional group so if the carboxyl is negatively charged, these charges may cancel out. Hence it may be less polar under these conditions and retain less by ANP. Try methods with formic acid, acetic acid, TFA, or other acid additive.

Click *HERE* for Diamond Hydride™ HPLC column ordering information

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