

**Develop Stable Methods
 Using 100% Aqueous Mobile Phase**

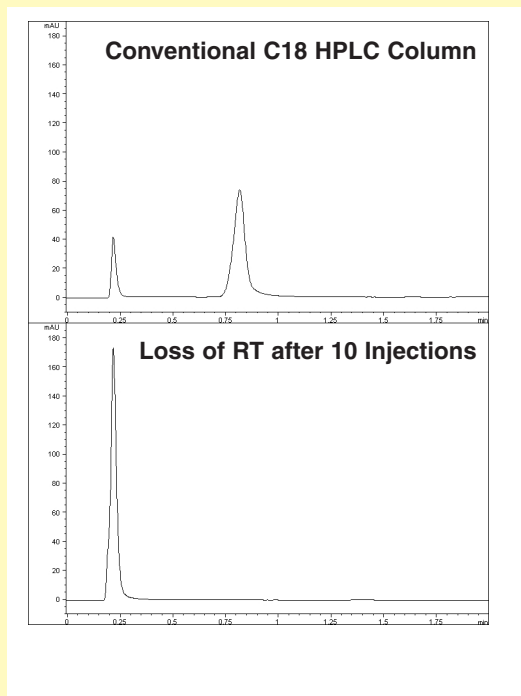
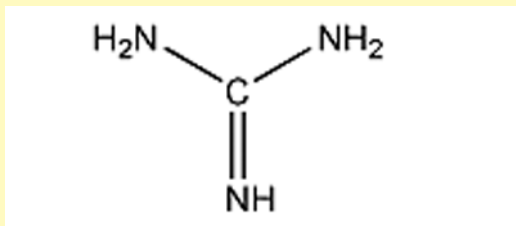


Figure 1: Separation of guanidine under conditions shown using C18 based on ordinary silica in reversed phase. Note the loss of retention after only about 10 injections. This conventional column produced poor peak shape with low efficiency, with less retention than a Bidentate C18™ column used in reversed phase.

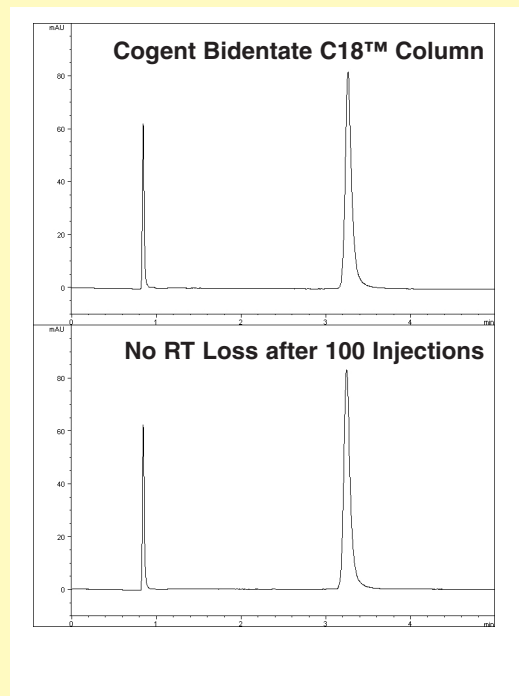


Figure 2: Above shows a separation of guanidine using the same method conditions, but with a Cogent Bidentate C18™ (L1 column). The upper chromatogram is the initial separation, and the lower after 2 days of use with over 100 injections. No changes in retention were observed. Note the excellent stability of the type C phase under very acidic mobile phase conditions that would hydrolyze other reversed phases. See next page for conditions.

A Stable Method Using 100% Aqueous Mobile Phase Cleaning Validation of Guanidine

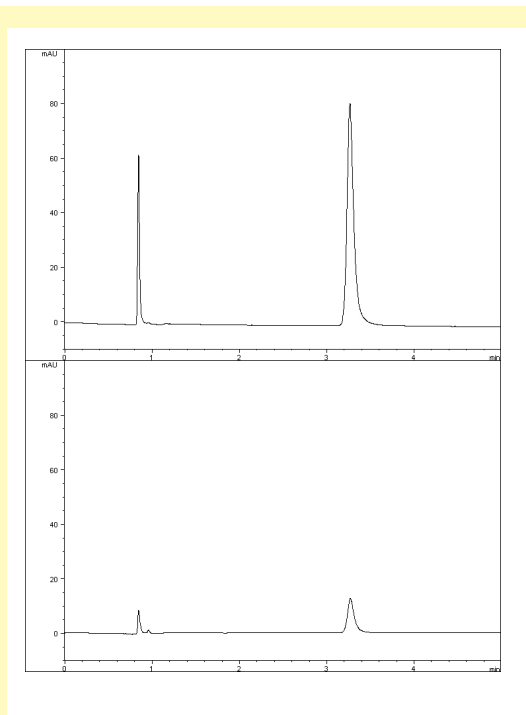


Figure 3: Cleaning validation example for residual guanidine. Upper chromatogram shows a standard at about 100 ppm, lower shows a real life sample with a residue of about 10 ppm.

Note: It may be necessary to remove guanidine from protein preparations, especially if they are to be used for therapeutic applications. Thus it is necessary to monitor low concentrations of guanidine. The Bidentate C18 offers a stable, reliable method that produces low LOQ compared with ordinary silica based columns.

Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100Å
Catalog No.: 40018-15P
Dimensions: 4.6 x 150 mm
Mobile phase: 100% DI water + 0.5% phosphoric acid + 1.5g/L pentane sulfonic acid
Flow rate: 1.0 mL/minute
Peaks: 1: Impurity
2: Guanidine
Injection Volume: 20 µL
Detection: UV 200 nm
Temperature: 25°C
Std Concentration: 100ppm
Sensitivity: ~1.6ppm as LOQ

Discussion

Guanidine is a strong base, therefore it will be protonated at all pH values below 12 thus will carry a positive charge. The low molecular weight of guanidine, with its positive charge, and lack of a significant chromophore, make the analysis very difficult. Since guanidine is such a polar compound, it requires a 100% aqueous mobile phase to be retained in reverse phase on a C18 column. Normally this mobile phase would require a specialty column such as a polar-embedded phase, but these columns are hydrolytically unstable and have other issues while running the method. Since the Cogent Bidentate C18 does not suffer from loss of retention from run to run which is commonly known as “phase collapse” and it is not a specialty column, it can be used as an L1 or reversed phase C18 column. One of the advantages of this column is that it is hydrolytically stable under aggressive, acidic mobile phase conditions as shown in this application note. Also, since it is still an L1 column, it could be interchanged with a C18 method column without revalidation of the method. This separation works best with a highly acidified mobile phase.

For more information visit www.MTC-USA.com

Cat. No.	Description
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40018-15P	Cogent Bidentate™ C18 HPLC Column, 4mm, 100A, 4.6mm x 150mm
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