COGENT 2.0 UDA™

Ion Exchange Application

Extended Application Note

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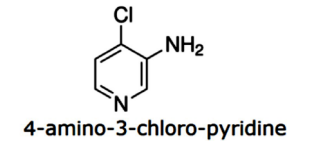
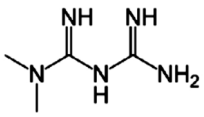
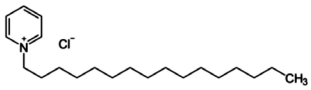


4-amino-3-chloro-pyridine



metformin



cetylpyridinium chloride

Figure 1

Method 1:

Time (min.)	%B1
0	95
1	95
17	40
19	40
20	95

Method 2:

Time (min.)	%B2
0	100
1	100
17	40
19	40
20	100

Post Time: 5 min
Flow Rate: 0.3 mL/min
Injection Volume: 0.5µL
Detection: UV 254 nm

Introduction

Ion exchange chromatography can be a useful tool available to the analytical chemist. In this separation mode, an ionized moiety on the stationary phase interacts electrostatically with an oppositely charged analyte. There are several types of ion exchange that can be used, such as strong anion exchange, weak anion exchange, strong cation exchange, and weak cation exchange. The Cogent UDA 2.o™ column uses weak cation exchange. The material consists of a C11 chain with a carboxylic acid group at one end. This group can either be neutral or ionized depending on the mobile phase pH. Under acidic conditions, the carboxylic acid is protonated and neutral. At near-neutral pH and above, the moiety becomes anionic and ion exchange is possible.

All TYPE-C Silica™ based HPLC columns can be used in the Aqueous Normal Phase (ANP) mode, in which analytes are retained on the basis of polarity. However, the Cogent UDA 2.o™ column has the additional advantage of retention by ion exchange. This can impart additional selectivity to a separation in which ANP chromatography may be insufficient to achieve full resolution.

We investigated the ion exchange characteristics of this column using three test solutes that illustrate its benefits in terms of chromatographic separation. The structures of the three analytes are shown in **Figure 1**. Each analyte contains amine functional groups which would be suitable for ion exchange interactions with the UDA carboxylate moiety.

Experimental

Materials

Metformin and cetylpyridinium chloride reference standards were from the United States Pharmacopeia (Rockville, MD, USA). 4-amino-3-chloro-pyridine was from Matrix Scientific (Columbia, SC, USA). Formic acid (FA) was from EMD (Gibbstown, NJ, USA). Deionized water (DI H2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (ACN) (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

A Hewlett–Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector was used. The system was interfaced with Agilent Chemstation (Santa Clara, CA, USA) software. The analytical column was 2.1 mm (i.d.) x 50 mm and packed with a Cogent UDA 2.o™ stationary phase (MicroSolv Tech. Corp., Leland, NC, USA). The particle diameter was 2.2µm and the pore size was 120Å. The binary mobile phase solvents were A: DI water + 0.1% formic acid, B1: acetonitrile + 0.1% formic acid, and B2: 95/5 acetonitrile/ 10mM ammonium acetate. The gradient programs are shown to the left.

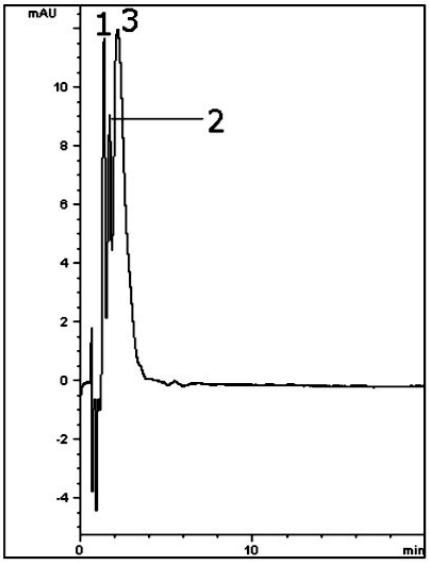


Figure 2

1. Cetylpyridinium chloride
2. 4-Amino-3-chloro- pyridine
3. Metformin

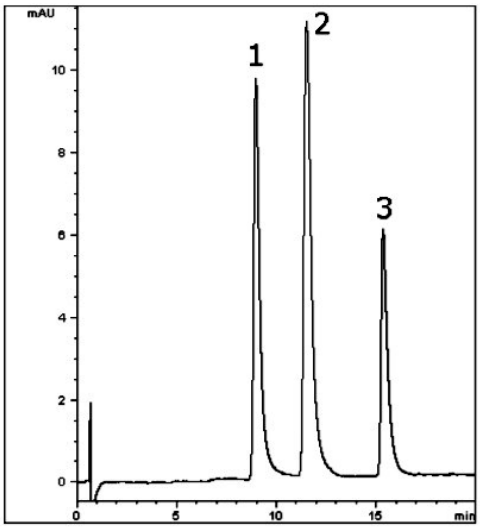


Figure 3

1. 4-Amino-3-chloro- pyridine
2. Metformin
3. Cetylpyridinium chloride

Sample Preparation

Stock solutions of each analyte were prepared at 1.0 mg/mL concentrations in a diluent of 80/20/0.1 acetonitrile/DI water/formic acid. Dilutions of each stock solution were prepared a 0.1 mg/mL concentrations (to determine elution order). A mixture was prepared with concentrations as follows: 0.02 mg/mL 4-amino-3-chloro-pyridine, 0.2 mg/mL metformin, and 0.1 mg/mL cetylpyridinium chloride.

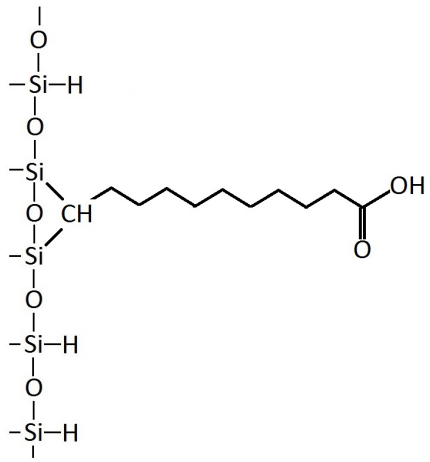
Results and Discussion

Initially, 0.1% formic acid was selected for both A and B solvents in the gradient (**Method 1**). Here, the UDA carboxyl group would be neutral and therefore any retention will be predominantly due to ANP behavior. The data for this separation is shown in **Figure 2**. All three analytes elute near the same time in the gradient, resulting in no separation. In this instance, it appears that a secondary retention mechanism may be useful in achieving additional selectivity. Therefore the weak cation exchange properties of the column were then explored.

During method development, a 10 mM ammonium acetate additive was used in both the A and B solvents in order to keep the carboxyl group de-protonated so that ion exchange could occur. However, it was observed that cetylpyridinium chloride did not elute under any gradient conditions in this case (data not shown). Since it has a permanent positive charge, this behavior would be expected in ion exchange mode. For this reason, a 0.1% formic acid additive was used in the A solvent only (**Method 2**). Here a pH gradient is used in which the method starts out with ammonium acetate and formic acid is gradually introduced. The data using these conditions is shown in **Figure 3**. Separation of all three test solutes is obtained. Interestingly, the elution order is even different than the formic acid method. A change in elution order is often indicative of a different retention mechanism, which we may postulate was ion exchange in this case.

Conclusion

An understanding of how the Cogent UDA 2.o™ column works is essential for obtaining good results. A combination of both ANP and weak cation exchange characteristics are believed to be at work. If analysts obtain data similar to that of Method 1, where analytes are poorly retained and not separated, they should consider operating at a pH where ion exchange is possible. Under the right conditions, excellent separation can be achieved.



For more information visit www.mtc-usa.com

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