Robust Methods with High Efficiency, Bonded-Carbon HPLC Columns

Technical Bulletin #268

Elena Khmelnitskaia
Cabot Corporation

Dwight Stoll, Peter W. Carr
ZirChrom Separations, Inc.

Loss of stationary phase, retention drift, and short column life are common problems using bonded-phase silicas, especially with aggressive mobile phases. A new bonding technology overcomes these problems by attaching C18 groups to a carbon surface with ultra-stable carbon-carbon bonds. This note shows that robust methods with high efficiency are now possible using ultra-stable bonded-carbon columns.

Introduction

The long-term reliability of an HPLC method depends greatly on the ruggedness of the stationary phase. In bonded silicas, an Si-O-Si bond is used to attach functional groups to the silica surface. It is well-known that this bond is subject to chemical attack, especially at low pH. The silica itself dissolves readily in aqueous mobile phases at high pH. Even sophisticated silica bonding technologies have not solved this problem[1]. The basic instability of bonded silicas causes retention drift, short column life, and frequent replacement of the column and re-qualification of the HPLC system. This is expensive both in terms of actual expenditures and in terms of lost productivity.

Bonded-Carbons

Zichrom Separations, Inc. and Cabot Corporation have developed new materials using unique technology to bond functional groups directly to the surface of carbon. The surface bond is C-C, which is extremely resistant to chemical and thermal attack. The authors have run mobile phases at very high pH (1M NaOH), very low pH (0.5M HNO_3), and at elevated temperature (up to 200 ^oC), and have not observed loss of bonded ligands.

Experimental

A method reliability test was set-up using 480 injections of a barbiturate mixture. A single DiamondBond™-C18 column (4.6 mm x 100 mm) was used for all of the injections. A new mixture of analytes was prepared after each 100 injections (analytes were purchased separately from Alltech). New mobile phase (10/15/75 THF/ACN/20 mM Ammonium Phosphate, pH 7.0) was also prepared fresh after each 100 injections.

Results

The results show that the separation has excellent long-term stability. Table 1 shows the average retention times for the analytes and the standard deviations. The relative standard deviations are generally 2% or lower.

Table 1 – Reproducibility of Barbiturate Method

Analyte	Avg. k'	St. Dev.	Relative St. Dev.
Barbital	0.46	0.011	2.4%
Metharbital	1.12	0.021	1.9%
Butethal	2.54	0.024	0.9%
Hexobarbital	3.18	0.044	1.4%
Mephobarbital	4.27	0.029	0.7%
Pentobarbital	4.92	0.063	1.3%

Figure 1 shows chromatograms for the first, 100th, 400th, and final injection. Note that this improvement in ligand stability also helps with LC/MS separations, since there is no ligand bleed to create noise in the MS baseline. ZirChrom's technical support group has extensive experience in this area, and would be happy to help you with your particular application.

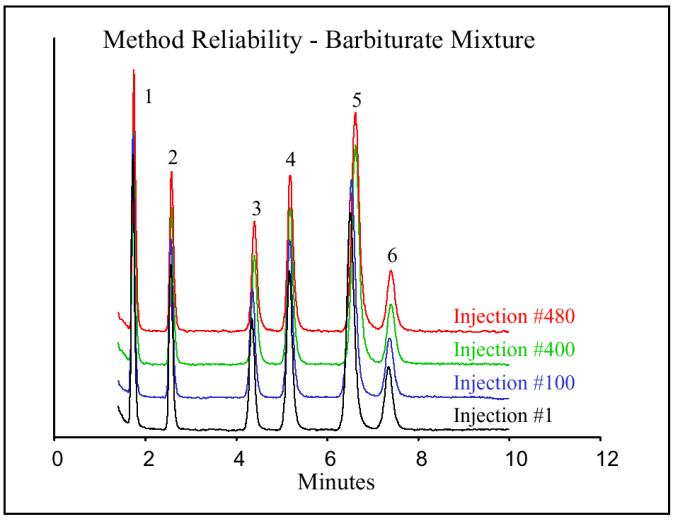

Figure 1: Barbiturate Method Reliability
1=Barbital, 2=Metharbital, 3=Butethal, 4=Hexobarbital, 5=Mephobarbital, 6=Pentobarbital

References

(1) J. J. Kirkland et. al., Anal. Chem. 61, 2-11 (1989).

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency DiamondBond columns from ZirChrom.