Recent Developments in Type C Stationary Phases:

Exploiting the Versatility of Silica Hydride Materials

by Joseph J. Pesek, Department of Chemistry, San Jose State University, San Jose, CA 95192, USA*
email: pesek@sjsu.edu • Tel: 1-408-924-4950 Fax: 1-408-924-4945
Maria Matyska, Microsolv Technology Corporation, One Industrial Way West, Eatontown, NJ 07724, USA
*Corresponding author**

TYPE C™ silica is a relatively new chromatographic material that has been finding ever-increasing use in the last few years. The properties exhibited by these stationary phases are often significantly different than the ordinary silica used for most commercial products. While all TYPE C phases can be utilized in the reversed-phase, organic normal phase and aqueous normal phase modes, there are some unique capabilities within each retention mode that have resulted in innovative method development strategies with great success. Some of the more challenging separation problems involve polar compounds; two approaches for the analysis of hydrophilic compounds are described in this report.

Introduction

TYPE C silica, based on a surface of Si-H, was introduced many years ago. However, it has only been recently that some of the unique chromatographic features of this material have been discovered and exploited in solving challenging separation problems. This report focuses on the capabilities of these stationary phases for the separation of hydrophilic materials in two modes: aqueous normal phase (ANP) that utilizes high organic content mobile phases and in reversed-phase using high aqueous content mobile phases. For descriptions about the chromatographic properties of TYPE C in the organic normal phase, earlier reports have provided examples of separations utilizing this separation mechanism [1,2].

The chromatographic retention and separation of polar compounds continues to be a challenging analytical problem. The versatility and ruggedness of reversed-phase chromatography for separations based on hydrophobic interactions has not been matched by any single method for hydrophilic species. A number of approaches have been developed for polar compound retention but many are limited in their applicability or have other serious drawbacks. For example polar compounds can be derivatized to make them amenable to RP methods, but this is often time-consuming or not very reproducible. Ion-exchange can be used for some polar compounds, provided they have a permanent charge, but is not applicable to neutral polar compounds like carbohydrates and is also not compatible with mass spectrometry, the most rapidly expanding method of detection. Making polar compounds neutral by the use of extremely high pH mobile phase and more recently, hydrophilic interaction liquid chromatography (HILIC) have been introduced as a means of analyzing polar compounds. However, many labs report that HILIC methods have poor reproducibility and systems equilibrate slowly when gradient elution is used. Also, many of the analytical schemes developed are not compatible with MS detection. Some of the problems reported for HILIC are likely related to the retention mechanism of these materials; generally regarded to be the formation of a water layer near the surface of the stationary phases so that polar molecules partition between it and the more organic-rich

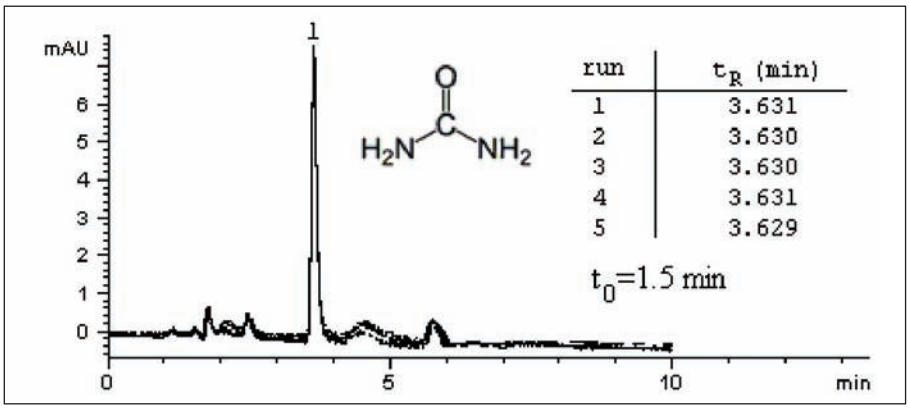

Figure 1. Analysis of urea in a 100% aqueous mobile phase on a Cogent Bidentate C18 column. Mobile Phase: 100% DI water (isocratic). Column: 4.6 x 150 mm. Flow rate: 0.5 mL/min. Detection at 210 nm. Sample: 1 mg/mL. Injection volume: 10 µL.

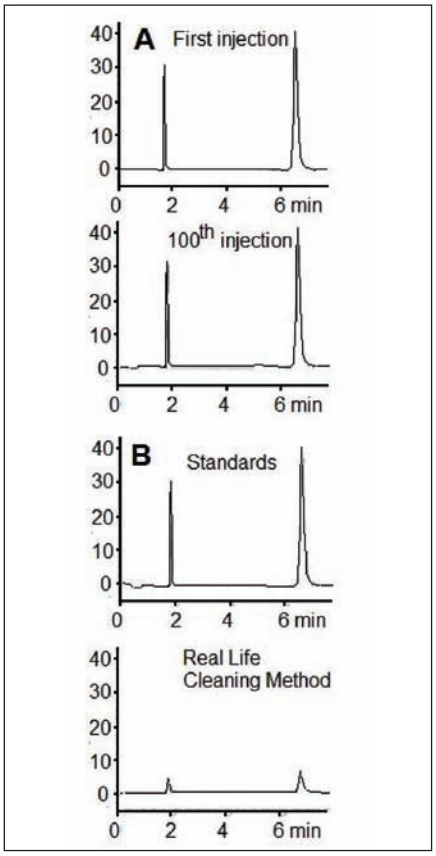

Figure 2. Analysis of guanidine in a 100% aqueous mobile phase on a Cogent Bidentate C18 column.

A. Repeatability of analysis using guanidine Standard.

B. Comparison of retention times of standard and real sample. Mobile Phase: 100% DI water + 0.5% phosphoric acid + 1.5g/L pentane sulfonic acid (isocratic). Column: 4.6 x 150 mm. Flow rate: 1.0 mL/min. Detection at 200 nm. Standard Sample: 100 ppm. Injection Volume: 20 µL.

surrounding of the mobile phase. TYPE C stationary phases are an entirely different material with a slightly more hydrophobic surface that does not generate a dense water layer at the particle/mobile phase interface. While the mechanism of separation is not yet completely understood, the actual retention and separation capabilities for hydrophilic compounds have been extensively demonstrated [3-7].

The most rapidly growing and the most extensively investigated area of polar compound retention is in the aqueous normal phase (ANP) mode. Under these mobile phase conditions the stationary phases have properties that are similar to the characteristics of HILIC phases (increasing retention with increasing amount of organic component, usually acetonitrile or acetone). In contrast to many HILIC phases and applications, the TYPE-C silica material is robust (lasts for hundreds of injections), very reproducible from run-to-run (% RSD values generally 0.5% or less) and equilibrates rapidly (five minutes or less) after a gradient method. Applications where the analysis of polar compounds is essential include plant, animal, human and drug metabolomics, clinical analysis, impurity testing, food safety and nutrition, forensics and environmental monitoring.

Reversed Phase

All TYPE C stationary phases display some reversed-phase behavior. Even the unmodified material can retain nonpolar compounds because the hydride surface is slightly hydrophobic. As the hydrophobicity of the stationary phase is increased by having greater surface coverage of bonded organic moieties, retention of nonpolar compounds increases as with all other reversed-phase materials. Selectivity for common phases such as C18 and C8 is somewhat different because of the underlying hydride surface and the resulting lack of water on this material.

For retention of hydrophilic compounds under RP conditions with TYPE C materials, mobile phases are used that typically contain 90-100% (v/v) water. Stationary phases based on silica hydride are especially suited to these conditions since they do not undergo "dewetting", sometimes referred to as phase collapse or phase fold back. In highly aqueous environments, many hydrophobic bonded phases such as C18 will try to minimize their contact with the polar environment by forming highly associated packets of organic moieties. This process reduces the total hydrophobic surface available for solutes to interact with and thus retention can drop drastically in high aqueous mobile phase environments. A few examples utilising high aqueous content mobile phases will be presented to illustrate this capability.

Figure 1 shows five overlaid chromatograms of a sample containing urea obtained on a C18 TYPE-C stationary phase using a 100% aqueous mobile phase. This highly polar compound is adequately retained under these conditions (k ≈ 1.5) demonstrating that the TYPE-C materials are particularly useful for hydrophilic compounds even in this mode. In addition, there is a remarkable degree of repeatability in these five runs which is another feature of the TYPE C stationary phases. Since this column material has an octadecyl bonded moiety, it can function like other reversed-phase stationary phases and can be used for the analysis of a wide range of hydrophobic compounds as well.

Another example of the retention capability for hydrophilic compounds in the reversed-phase mode is the analysis of guanidine shown in Figure 2. Guanidine is a strong base and is protonated at all pH values below 12. The low molecular weight of guanidine, its positive charge, and lack of a significant chromophore, make the analysis very difficult. Since guanidine is such a polar compound, it also requires a 100% aqueous mobile phase to be retained in reversed- phase on the TYPE-C C18 column. Normally thisanalysisanalysis would require a specialty column such as an ion-exchange phase to enhance retention of this polar molecule but there areother issues using this approach [8] including long run and re-equilibration times. Since the Cogent Bidentate C18™ (TYPE-C with C18 bonded moiety) does not suffer any loss of retention from run to run which is commonly known to be due to dewetting or "phase collapse" and it is not a "specialty column", it can be used

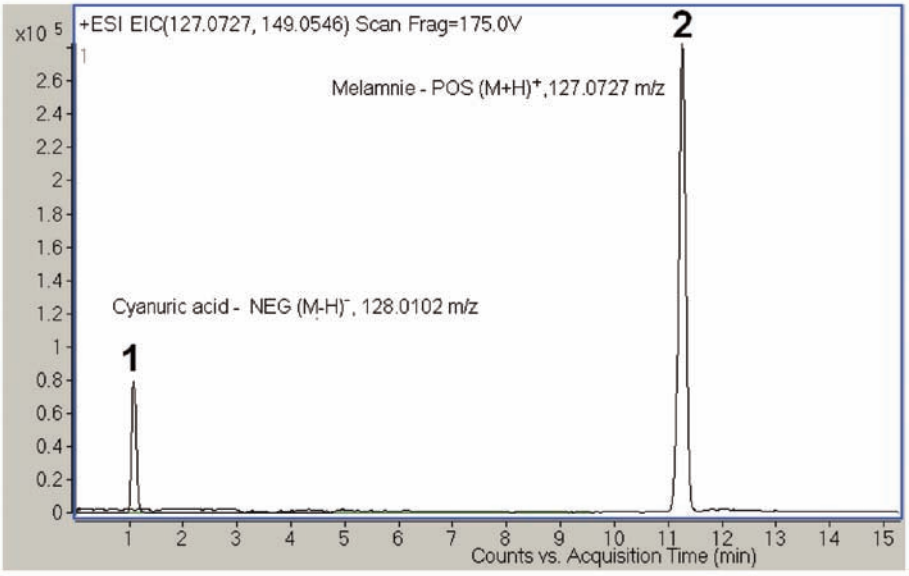

Figure 3. Separation of melamine and cyanuric acid on the Cogent Diamond Hydride column using an acetic acid mobile phase and a gradient from high to low concentration of acetonitrile in the mobile phase. Mobile Phase: A: DI water + 0.1% acetic acid; B: Acetonitrile + 0.1% acetic acid. Gradient: 100% B to 50% B in 15 min. Column: 2.1 x 150 mm. Flow rate: 0.4 mL/min. Sample Concentration: Cyanuric acid 1.5 µg/ml and melamine 3 ug/mL. Detection: m/z 128 in the negative ion mode for cyanuric acid and m/z 127 in the positive ion mode on an Agilent 6210 MSD TOF spectrometer.

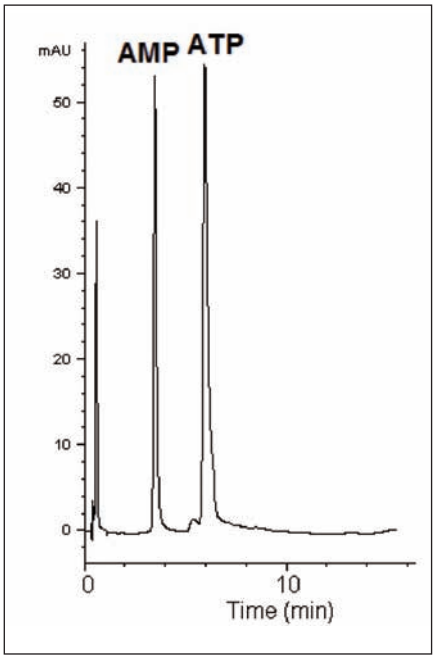

Figure 4. Gradient separation of AMP and ATP on a Cogent Diamnond Hydride column. Mobile Phase: A, DI water + 0.1% ammonium formate; B, 90:10 acetonitrile/DI water + 0.1% ammonium formate. Gradient: 95% B to 70%B in 10 min. Column: 2.1 x 100 mm. Flow rate: 0.3 mL/min. Sample concentration: 0.3 mg/mL of each. Detection @ 254 nm.

as an L1 or reversed phase C18 column for USP methods. In Figure 2A the reproducibility of the method is shown where after 100 injections the retention time of guanidine (second peak) is essentially the same as the first injection. In Figure 2B is the analysis of the residual guanidine removed from a protein preparation and the retention is compared to a standard sample. Such a procedure is common if the protein is to be used for therapeutic applications. One of the advantages of this column is that it is hydrolytically stable under aggressive, acidic mobile phase conditions as shown in this application. Also, since it is still considered an L1 column, it could be interchanged with a C18 method column without revalidation of a validated GMP method.

Aqueous Normal Phase

Every TYPE-C stationary phase displays ANP properties, i.e. increased retention of hydrophilic species as the amount of the least polar component in the mobile phase increases. The mobile phase consists of water and typically either acetonitrile or acetone. Thus it is very easy to transition (no hysteresis) from RP to ANP since water is the common mobile phase constituent in each mechanism and the attraction of water to the particle surface is weak and desorbs very easily. In general, the extent of ANP retention is dependent on the amount and type of modification of the particle surface. Maximum retention of hydrophilic species under ANP conditions is obtained for the totally unmodified or minimally modified surface while lesser, but still appreciable, polar retention is obtained even when C18 or C8 bonded groups are present.

The retention of polar compounds in the ANP mode is best illustrated by some examples that utilize the Cogent Diamond Hydride™ (DH) stationary phase. This phase, which has a small amount of carbon on the surface, has both high hydrophilic retention as well as excellent peak shape over a wide range of polar compounds. An analytical problem that seems to continually make the news is the determination of melamine and its degradation product, cyanuric acid, which have been found to be contaminants in both human and animal food sources. Figure 3 shows the analysis of these two compounds using LCMS with the DH stationary phase. The detection of cyanuric acid is done in the negative ion mode while melamine is detected as a positive ion. Using a TOF MS system the sensitivity is in the nanogram range while with an MS/MS system the detection limit is sub-nanogram. As can be seen in Fig. 3, the separation of these two compounds is substantial with both peaks having excellent efficiency and peak shape.

An important metabolic determination is the analysis of adenosine monophosphate (AMP) and adenosine triphosphate (ATP). Nucleotides are important phosphate containing compounds that are found in living cells and are associated with a broad array of metabolic and biological processes. They have significant roles in the synthesis of DNA and RNA, are involved in signal transduction pathways, function as coenzymes in biosynthetic pathways and serve as energy reservoirs in biological systems. Figure 4 shows an example of the separation of the two adenosine analytes using an ANP gradient method. The gradient starts at a high percent of acetonitrile in the mobile phase and goes to 70% (v/v) over 10 min. The last component, adenosine 5'-triphosphate (ATP) has some tailing in comparison to the first components. An improvement in peak shape can be achieved by adding a small amount of ammonia to the sample. The amount used here (5 µL of 12% ammonia/mL) does not appreciably affect the retention times.

Finally, two examples of pharmaceutical analyses will be described to document the retention of polar compounds on TYPE-C-based columns having reasonably high surface coverages of larger bonded molecules. Metformin, a drug used in the treatment of diabetes, was analyzed on a Bidentate C18 column [9]. A mobile phase of acetonitrile and DI water with 0.1% formic acid is used for the analysis as is the case for many basic compounds. Retention begins to increase at 60% acetontrile, becomes substantial at 70% and has a k (retention factor) greater than 5 when the amount of acetonitrile reaches 80%. An even more basic drug, Tobramycin, an antibiotic has been analyzed on a TYPE-C-based UDC-Cholesterol™ column [10]. The additive again is formic acid but an even more unusual aspect of this analysis is that methanol as well as acetonitrile can be used as the organic component of the mobile phase. This situation is relatively rare since in most cases methanol is generally too strong of a solvent to produce significant retention in the ANP mode. But in the case of some highly polar compounds, methanol becomes a viable solvent for ANP. Naturally retention is considerably less than with acetonitrile or acetone.

References

1. *J.J. Pesek, M.T. Matyska, A. Sharma, J.Liq. Chromatogr. & Rel. Technologies, 31 (2008) 134.*
2. *J.J. Pesek, M.T. Matyska in "Advances in Chromatography, Vol. 48", E. Grushka, N. Grinberg, eds., CRC Press, Boca Raton, FL, 2010, pp. 255.*
3. *J.J. Pesek, M.T. Matyska, S.M. Fischer, T.R.Sana, J. Chromatogr. A, 1204 (2008) 48.*
4. *J.J. Pesek, M.T. Matyska, M.T.W. Hearn, R.I. Boysen, J. Chromatogr. A, 1216, (2009) 1140.*
5. *J.J. Pesek, M.T. Matyska, J.A. Loo, S.M. Fischer, T.R. Sana, J. Sep. Sci., 32 (2009) 2200.*
6. *J.J. Pesek, M.T. Matyska, J. Sep. Sci., 32 (2009) 3999.*
7. *J.J. Pesek, M.T. Matyska, J. Duley, M. Zamzami, S.M. Fischer, J. Sep. Sci., 33 (2010) 930.*
8. *J. Qui, H. Lee, C. Zhou, J. Chromatogr. A, 1073 (2005) 263.*
9. *J.J. Pesek, M.T. Matyska, LCGC, 24 (2006) 296.*
10. *http://www.microsolvtech.com/hplc/app_udc_amgly.asp*