WHITEPAPER

The Method Development Advantages Offered by Hybrid-Silica Products

By Alissa Marrapodi

Separation sciences such as chromatography continue to be the common method of analysis, characterization, purification, separation, and general preparation. The stationary phase is arguably the largest single factor that impacts separation factor (selectivity), but it often takes a backseat to efficiency.[1]

For many years, traditional silica was the standard base material used to create stationary phases for chromatographic columns. But there is limited stability in silica-based stationary phases in neutral- to high-alkaline mobile phases, which is caused by increased solubility at high pH. In the review "*Silica, Hybrid Silica, Hydride Silica and Non-Silica Stationary Phases for Liquid Chromatography*", Brazilian researcher Endler M. Borges said, "Free silanols on the surface of silica are the 'villains', which are responsible for detrimental interactions of those compounds and the stationary phase (i.e., bad peak shape, low efficiency)."[2] As a result of silica's limitations, hybrid silicas, which reduce and/or shield silanols, were introduced into the market.

"Hybrid silica particles became more prominent for two reasons," said Joseph L. Glajch, Ph.D., principal consultant, JLG AP Consulting LLC. "When they first came out, there was a reluctance because of the long history with silica, and people knew it could be made reproducibly. But now that they've been around for a while, they are more accepted. The second reason is some of the molecules we are looking at now are more basic—for whatever reason, they are different from the molecules from many years ago. Today we see some materials that could be separated better at a higher pH."

Many laboratories are turning to higher performance hybrid silicas because of their advantages over the traditional form. "Hybrid silicas were developed to extend the pH range of silica particles," Glajch said. "The older silica particles that we used many years ago were only good between pH 2 and 7. As the technology has gotten better, they've extended to 8 or 9. If you were looking for higher pH materials, before hybrids were developed, the only option you had was polymers."

Advantages of Hybrid Silica

There are several advantages of hybrid silica over traditional silica. First, it offers more stability for mechanical robustness (sub-2 µm particles for ultrahigh-pressure liquid chromatography [UHPLC]), improved chemistry (due to two aspects of inorganic and organic character), and more uniform and precise structure.

"Hybrid silica can sometimes provide unique selectivity or better sensitivity and resolution," said Michael Swartz, Ph.D., member of *LCGC* Editorial Board and pharmaceutical consultant.

According to a review published in the *Journal of Chromatographic Science*, second-generation hybrid silica, Type C silica, PGC, and zirconia polymer-coated stationary phases give chromatographers the tools they need to work with a wider range of experimental design, i.e., temperature and pH, and open the door to mobile phases using buffers and solvents, such as alkaline eluents, that were not an option before.[3] "These columns, therefore, make it much easier to achieve desired chromatographic selectivity or resolution targets for a particular analytical problem," the researchers said. "They also provide reproducible separations at elevated temperatures under acidic conditions without column deterioration. The ability to perform analyses at low and high pH values as well as different temperatures considerably increases the likelihood of success when generic gradient programs are employed, for example in discovery applications and impurity profiling in pharmaceutical analyses."

These silica particles can improve analysis speed and peak resolution, and they require reduced solvent consumption. "By sometimes providing sharper peaks, impurities can be better resolved," Swartz said. "I've had the experience where a hybrid C18 charged surface chemistry provided 30 - 50% sharper peaks and less tailing than standard C18 chemistries, which can make the difference between running the existing method for new process impurities or degradants, or spending more time optimizing or redeveloping a method."

Hybrid silicas can also offer greater column-to-column reproducibility for identical results under known conditions; and they offer direct scaling from analytical to process use with fewer method changes. Even more important is their added flexibility of conditions, including a wider pH range of mobile phases than traditional silica.

"Oftentimes, [hybrid silica] allows for methods to be developed with mobile phases that are in the basic pH (7 to 12) range—regular silica can typically only be used at pH 2 to 7," explained Jeffrey A. Kakaley, applications and marketing manager for HPLC & UHPLC columns, YMC America. "It has a wide temperature range—up to 90°C in some cases. Hyrbrid-silica phases are typically better than the same types of phases based on [traditional] silica particles. Hybrid phases give biochromatographers more leeway to develop methods over a wider range of conditions."

In a German study, high-temperature reversed-phase LC was coupled to and evaluated for isotope ratio mass spectrometry with four different stationary phases.[4] XBridge C18 (up to 180°C), Acquity C18 (200°C), Triart C18 (150°C), and Zirchrom PBD were utilized under isothermal and temperature gradient conditions without column deterioration or column bleed. The review concluded that new hybrid stationary phases, special chemical bonding and end-capping, and zircoinia can be implemented at temperatures greater than 100°C with a non-buffered mobile phase.

Researchers also evaluated the pH stability and chromatographic performance of a novel C18 column (YMC Triart) based on organic/inorganic hybrid silica. They found the columns enhanced durability and chromatographic performance and offered maximum flexibility in separation conditions across an expanded pH range.[5,6] The 1.9 µm column with excellent chromatographic performance and 100 MPa maximum operating pressure enabled ultra-fast and reliable analysis. Additionally, identical chromatographic performance and selectivity across different particle sizes provided mutual method transfer among UHPLC, HPLC, and semi-preparative LC. The combination of the three core technologies produced a material with outstanding chemical and physical durability and also provided excellent peak shape for various types of compounds under a variety of mobile-phase conditions.[7]

In Application: Beta Blockers, Proteins, and Bioactive Compounds

As research has shown, hybrid silica is capable of analyzing at low and high pH values and different temperatures, which increases the likelihood of success in discovery and profiling.

As a result, hybrid silica columns are used in numerous industries to analyze, investigate, discover, and examine the constituents of various compounds and substances—from the pharmaceutical to the food and beverage industry.

During a case study published in *Microchemical Journal*, researchers detected three beta-blockers in human urine using a newly developed microextraction by packed sorbent (MEPS)-HPLC coupling and a multi-layered particle column for chromatographic separation.[8] A new on-line hyphenation of the modern micro-extraction procedure with LC was developed and used for human urine sample clean-up and pre-concentration of three beta-blockers—metoprolol, labetalol, and propranolol—before separation and determination. A commercial MEPS C-18 cartridge was coupled using Teflon holders into a HPLC system through a high-pressure six-port switching valve. Chromatographic separation was performed on a Triart YMC C-18 (50 mm × 4.6 mm) analytical column with 5 µm hybrid-silica particles. The hybrid-silica particle column showed extremely low flow-resistance (<4.1 MPa) at a flow rate of 1.0 mL min−1. As a result, a direct connection with a MEPS cartridge was possible. A mixture of acetonitrile and an aqueous solution of 0.5% triethylamine with acetic acid was used with pH adjusted to 4.5. Linear gradient elution was used. This mobile phase eluted beta-blockers from the MEPS mini column directly onto the analytical column where the separation was carried out. Metoprolol, labetalol, and propranolol were detected by a fluorescence detector. The developed on-line MEPS-HPLC coupling showed satisfactory validation results with recovery ranging from 94.0 - 104.7% and precision in the range of 0.6 - 9.5% for all beta-blockers.

In blood banks, platelets are treated with a pathogen-reduction technology (PRT) and stored for seven days. But the platelets' storage time, and PRTs, can have an impact on their releasate, which can potentially lead to adverse reactions after transfusion to patients. In January 2020, Spanish researchers analyzed the proteome of extracellular vesicles—which are biomarkers for many diseases—derived from platelet concentrates stored at different times to gain more information on the platelet concentrates' state at those different times.[9] Using LC-MS/MS with a silica-based reversed phase analytical column (as YMC-Triart C18), more than 700 proteins were identified, many of them described in different platelet scenarios and by different proteomic approaches. The results indicated a higher platelet activation state in platelet concentrates treated with a PRT upon storage, with differences evident even after four days of storage. Proteins upregulated on extracellular vesicles at day seven of storage could be suggested as biomarkers of platelet storage lesion.

And in a 2019 study, researchers investigated the yields of various bioactive compounds and examined the enzymatic (alpha-amylase and glucoamylase) inhibitor activities of the stems of brown seaweed (*Undaria pinnatifida*) using conventional liquid solvent extraction methods and supercritical carbon dioxide (SC-CO2) fluid extraction.[10] The SC-CO2 with ethanol extraction produced high amounts of phenolic and flavonoid compounds—fucoxanthin, epicatechin, and gallic acid. They were evaluated using a HPLC system and a hybrid silica-based ODS column (as YMC-Triart C18). Also, the extracts obtained by SC-CO2 with ethanol exhibited the most potent inhibitor of alpha-amylase and glucoamylase among all the extracts studied, perhaps because of the high content of fucoxanthin. These results suggest that stems of *Undaria pinnatifida* could have value as a raw material to extract these bioactive substances. The stems present the potential for use in food production, particularly to produce functional foods with bioactive compounds that are effective at reducing hyperglycemia.

In Discovery: Peptides, Oligonucleotides, and Chromatography

As drug discovery continues to create new biologics—drug products produced from living organisms such as peptides and monoclonal antibodies—and oligonucleotide therapeutics, hybrid silica plays a role in the advancement of these medical therapies.

Both peptides and oligonucleotides are the future of therapeutic and pharmaceutical intervention. “Several peptide drugs are essentially ‘replacement therapies’ that add back or supplement peptide hormones in cases where endogenous levels are inadequate or absent,” according to a study published in *Bioorganic & Medicinal Chemistry*.[11] One of the first examples of this is the use of insulin in the 1920s in diabetics who lacked sufficient quantities of the hormone. Oligonucleotide therapeutics, such as antisense, siRNA, aptamer, and so forth, are small strands of DNA (noting that nucleotides compose DNA). These therapeutic molecules are used in genetic testing, forensics, and other types of research.

Since the development of biopharmaceuticals, an effective analytical method with higher sensitivity, superior selectivity, and increased speed is needed in the characterization of peptides, proteins, and oligonucleotides by HPLC.[12] Method development of reversed-phase HPLC requires optimization of several conditions, such as the bonded-phase, column efficiency, solvent type, pH, and temperature. pH and buffer type are the most important parameters to control retention of

biomolecules that have multiple ionic functional groups. Furthermore, temperature often becomes a key tool to achieve better peak shapes and resolution for larger molecular-weight compounds. Although silica-based reversed-phase columns have been widely used for biomolecule separations, they have low stability under alkaline conditions and a limited usable pH range. To improve the chemical stability at an expanded pH and temperature range, researchers studied example cases of efficient method development in bioseparations.

A study out of Japan examined the effect of increasing temperature on peak shapes and resolution in separation of peptides and proteins and the effective separation of oligonucleotides by using an ion-pairing mobile phase and optimizing temperature.[13] These research results offered two conclusions:

- Highly sensitive, selective, and reproducible HPLC methods can be developed with a novel reversed-phase hybrid column using pH and temperature as key tools for optimization, in the analyses of peptides, proteins, and oligonucleotides.
- Hybrid silica (as YMC-Triart) columns offer significant advantages for simple and rapid method development of a variety of biopharmaceutical compounds.

Researchers also examined an efficient analytical method for short oligonucleotides using a reversed-phase packing material with a hydrophilic surface (as Hydrosphere C18, from YMC America) with an ion-pairing buffer and high-temperature analysis of oligonucleotides using a hybrid silica (as YMC-Triart C18).[14] The researchers:

- Compared d(pT)2-20 separation among commercially available C18 phases
- Examined the effect of composition and salt concentration of an ion-pairing mobile phase on separation and intensity of oligonucleotides
- Examined the effect of mobile phase and column temperature on separation of siRNA duplex
- Examined the purification of a crude synthetic oligonucleotide with dibutylamine-acetic acid (DBAA) buffer

The results showed the Hydrosphere C18 and Triart C18 phases, which are designed to have moderate hydrogen-bonding capacity, provided adequate retention and separation of oligonucleotides, even at low concentrations of ion-pairing reagent.

Triart C18 was applicable for denaturing HPLC—a technique that utilizes high temperature to generate single-stranded RNA and is widely used in the field of gene mutation analysis—analyzed at a high temperature due to its outstanding thermal stability.

From blood banks to food labs, hybrid-silica columns are enhancing HPLC and LC-MS/MS. And as biotherapeutics and oligonucleotides burgeon in drug discovery, separation sciences must continue advancing to meet the unique methods of analysis, characterization, purification, and separation for therapeutic molecules.

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