

FROM DISCOVERY TO PRODUCTION: HYBRID SILICA CHROMATOGRAPHY AND THE PHARMACEUTICAL INDUSTRY

CHROMATOGRAPHY IN DRUG DEVELOPMENT

Chromatography, which is the predominant separation science, plays a pivotal role in all stages of drug development. Traditional drug discovery focuses mainly on small molecules, but many biotechnology companies have become interested in larger therapeutic molecules, or biologics, such as peptides and monoclonal antibodies. Though the challenges may be different, small-molecule medications and biologics are both likely to have passed through a column on their way from bench to bedside. And as the pharmaceutical industry has changed and advanced, so too has chromatography, moving from sand and traditional silica supports to newer hybrid silica offerings.

From the discovery phase all the way through manufacturing, scientists execute many analytical and preparative separation tasks. For analytical purposes, researchers turn to chromatography to separate a mixture of compounds in a drug substance or formulation and identify and quantify the components. To that end, analytical chromatographers are mainly focused on stationary-phase selectivity, speed of separation, method sensitivity, and column reproducibility and lifetime. For preparative purposes, chromatography is used to separate and purify the active components of the mixture.¹ In contrast to their analytical brethren, preparative/process scientists are more concerned about loading capacity, mechanical strength, chemical durability (stability), and purity or recovery of the drug product. For both analytical and preparative chromatography, choosing the appropriate separation technique and stationary phase is critical to the separation success.

The major chromatographic technique used in the pharmaceutical industry is high-performance liquid chromatography (HPLC); a related method, ultra-high-performance liquid chromatography (UHPLC), is an updated platform that uses higher pressure. These methods are heavily used in drug production and development for quality control, to test the purity of pharmaceuticals.



YMC offers a variety of hybrid silica-based HPLC columns (YMC-Triart columns) for both analytical and preparative applications.

Image credit: YMC America, Inc.

Quality control: Identifying impurities and degradants

“The question of drug quality becomes critical during development,” says Michael Dong, principal consultant at MWD Consulting in Norwalk, Connecticut, and a subject matter expert in HPLC and pharmaceutical analysis. “HPLC is the primary technique to measure the impurities and quantify the active pharmaceutical ingredient.” Regulators require such HPLC measurements to ensure the quality of pharmaceutical products before they are introduced to the market.²

During synthesis, pharmaceutical compounds are inevitably produced with some impurities, says Jeffrey A. Kakaley, marketing and applications manager for HPLC & UHPLC Columns at YMC America, Inc. Over time, the drugs can also degrade, introducing additional safety hazards, Kakaley says, adding, “The degradation products have chromatographically different properties, and they can typically be separated and quantified.”

To assess the stability of a drug and identify its degradation products, chemists in the pharmaceutical industry typically develop a specialized HPLC method called a stability-indicating assay.³ Degradation products from forced degradation studies are run via the stability-indicating HPLC method, which separates those degradation products from the active pharmaceutical ingredient (API) and other impurities. “When it comes to drug development, purity equals safety,” Dong emphasizes. “As such, the resolution of the HPLC columns used is a critical factor to consider. The higher the resolution, the better the accuracy expected from your stability-indicating assay.”

For example, the resolution provided by the HPLC column will impact the number of peaks in the chromatogram. A low-resolution column may not show as many peaks for impurities or degradants. “You can improve the resolution by getting longer columns,” Dong explains. However, that can slow separation. “To increase process efficiency, it’s important to employ a high-quality, high-resolution column.”

The long game: Reproducibility and reusability

When manufacturing a particular drug over decades, pharmaceutical companies must maintain consistent separations, and columns must offer reproducible separations. Researchers often reuse columns many times over. HPLC columns are not trivial purchases; the average cost ranges from \$500 to \$1,000. To be cost effective, the column must be robust and able to operate efficiently and reliably under various conditions (pH, temperature, etc.) over a long lifetime. Thus, the reusability and robustness of the column will translate to considerable savings over time.

TRADITIONAL SILICA SUPPORT: ADVANTAGES AND DISADVANTAGES

Silica has been used in liquid chromatography since the early 20th century. The first silica used in liquid chromatography was sand, which consists of silicon dioxide and other metallic oxides. Scientists would sift the sand to get relatively uniform particles that could be packed into rudimentary columns. But the sand particles were still fairly irregular in size, and porosity could not be controlled.

Nowadays, silica gels with controlled porosity are made synthetically by the polymerization of purified tetraethoxysilane (TEOS).⁴ These silica gels have become the gold standard of chromatographic support for several reasons:

- The rigidity of the silicon dioxide networks imparts stability and tolerance to high pressure.
- Silica is inexpensive.
- Silica is easy to handle and modify.
- Silica is relatively inert.

Despite these advantages, traditional silica supports have limitations, explains Takashi Sato, sales and marketing manager of YMC Japan. “Silica doesn’t tolerate very high or very low pH.” Basic solutions cause silica to dissolve, while acidic conditions (pH < 2) can strip off the bonded phase.⁵

“Silica contains free silanol groups on the surface, even after surface modification,” Dong adds. “The residual silanols can react with basic (alkaline) drug components running through the silica-based columns to give undesired retention and ‘peak tailing,’ which is a pretty big hindrance in separation.” Dong further explains that peak tailing in a chromatogram is a shift from the symmetrical, Gaussian peak shape that results in the second half of the peak being wider than the first half. In the pharmaceutical industry, peak tailing is defined by the tailing factor (T_f).⁶ High tailing factors can cause a reduction in peak height and a miscalculation of the peak area. Particularly, in a stability-

indicating assay, a minor peak could hide under the tail of a major peak and easily be missed. “In such cases, the stability-indicating assay would fail to meet pharmaceutical regulations,” Dong says.

Residual silanol groups in silica supports are not all equal; they have different microenvironments. For instance, peak tailing is particularly serious when residual silanols are adjacent to metallic oxides. Older silica columns contained a high metallic content, leading to significant peak tailing. In such cases, researchers had to use mobile phase additives like triethylamine to obtain a satisfactory peak shape.⁷ Now, products are available that minimize the metallic sites and the active silanol groups to provide sharper peaks, Dong says. However, 100% removal of residual silanols is virtually impossible. “It is always a weakness of silica,” Dong notes.

WHAT HYBRID SILICA CAN OFFER

To address the limitations of traditional silica and impart improved chemical stability to HPLC, companies developed hybrid silica products. Hybrid silica contains carbon entities within the silicon dioxide network to make the support material more robust than traditional silica as well as resistant to a wider pH range.

The initial concept of a hybrid silica support was put forth in 1979,⁸ however, the actual commercialization didn't occur until the late 1990s. The hybrid silica in first-generation columns was synthesized by polymerizing a mix of monomers (tetraethoxysilane and methyltriethoxysilane in a ratio of 3:1).⁹ The addition of the methyl group into the silicon dioxide network created the hybrid, with one-third of the free silanols being replaced by methyl groups, which are chemically unreactive toward basic analytes.¹⁰

“In HPLC method development, particularly for basic pharmaceutical compounds, the ability to extend the pH of the mobile phase was fairly important, and both low-pH-stable and high-pH-stable hybrid columns were developed,” says Ronald E. Majors, column editor emeritus at *LCGC* magazine and consultant at ChromPrep. “Traditionally, silica-based bonded phase columns had a somewhat limited high pH range of around pH 8–9 and a low pH limit of pH 2, which became a significant challenge for separating alkaline drug compounds.”

Second-generation hybrid products, such as YMC-Triart hybrids, use bridged ethylene hybrid technology, where an ethylene-bridged comonomer is distributed throughout the silica backbone to create a highly cross-linked network.¹⁰ The degree of cross-linking is higher than in first-generation hybrid silicas, resulting in greater mechanical strength. Additionally, the Si-CH₂-CH₂-Si units within the network are more chemically stable and hydrophobic, leading to enhanced hydrolytic stability.

“Most hybrid products in the market today, including the YMC-Triart hybrids, use the ethylene linkers in the silicon dioxide network,” Sato says. “The mechanical stability is still quite intact and the hybrids are just as easy to handle and modify as the traditional silica. Basically, hybrid silica gels combine the advantages of both silica (mechanical stability) and a polymer (chemical stability).”

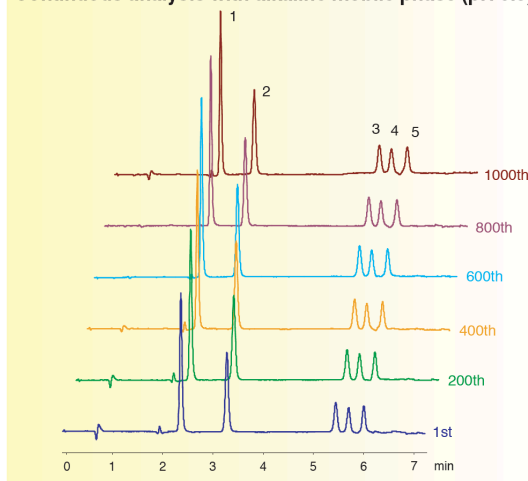
Although the common focus of hybrid silica manufacturers is to improve chemical stability, each product has unique properties. For example, YMC-Triart hybrids are designed to provide high batch-to-batch, column-to-column reproducibility during drug development, good peak shape for all types of drug compounds, and high loadability, or column capacity, for preparative purification processes, in addition to pH stability. Another key feature of YMC-Triart materials is that they can be directly scaled from the analytical scale (3 or 5 μm particle size) up to preparative/process scale (10 μm and greater particle size) with little to no further method development necessary. Selectivity is virtually the same across all particle sizes.

“YMC was founded in 1980,” Kakaley says. “Since that time, YMC has been meeting the ever-increasing demand for new LC stationary phases by developing a wide range of products targeting both preparative and analytical pharmaceutical applications. Not a lot of companies offer both, especially considering almost all of our products are fully scalable from analytical to prep.” YMC-Triart resins are used globally by pharmaceutical companies for research and development, quality assurance, and production of new chemical entities or APIs.

APPLICATIONS OF HYBRID SILICA

When analyzing a basic drug, it often helps to run the column at a high pH. Such harsh conditions can be tough on the support, so durability is key. Unlike traditional silica, hybrids can withstand alkaline conditions, providing better peak shapes. Hybrid silica columns can withstand higher pressures and temperatures than conventional silica columns. Bruce Weber, a senior analytical chemist at Hovione, and his team routinely use hybrid silica columns for the development of analytical methods to support the manufacture of APIs. Hovione focuses on producing off-patent APIs and is a major source of semisynthetic tetracyclines and corticosteroids.¹¹

Continuous analysis with alkaline mobile phase (pH 9.5)



Barbiturates

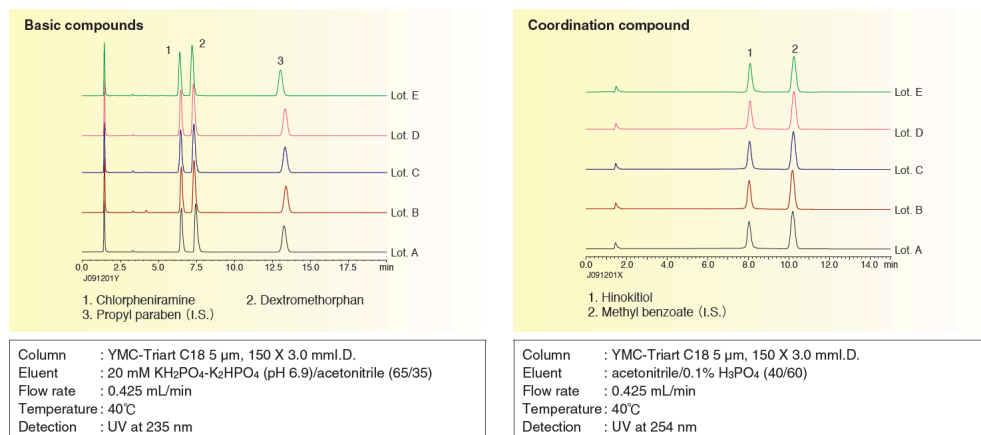
1. Barbital
2. Phenobarbital
3. Hexobarbital
4. Pentobarbital
5. Secobarbital

Even after the 1000th separation under an alkaline mobile phase, YMC-Triart columns show stable analysis, demonstrating that the columns are durable in high pH conditions over a long period of time. This is difficult to achieve with conventional silica columns.

Image credit: YMC America, Inc.

Weber once needed a method that could accurately quantitate a compound that existed as several rotameric forms at ambient temperatures. These rotameric forms caused the compound of interest, as well as its process impurities, to exhibit very broad and split peaks, and inhibited accurate integration. To obtain a sharp peak that could be accurately integrated, it was necessary to overcome the interconversion barrier between the rotamers. In this case, raising the column temperature and pressure to the upper limit of HPLC operation did the trick. “Even under these aggressive conditions, the YMC-Triart column provided good resolution and sensitivity for an extended period of time,” Weber says. “We were able to develop a usable and reproducible method that provided a baseline separation of all the components.”

Reproducibility is a key factor for quality control processes in the pharmaceutical industry. “We routinely screen a variety of columns when developing a method to analyze a novel pharmaceutical,” says Michael M. Puppolo, head of R&D analytical development at Hovione’s New Jersey site. “Hybrid silica columns are typically included in this selection because the hybrids are expected to provide improved reproducibility and column lifetime. When we find two different columns that give adequate selectivity and other required chromatographic characteristics for a given application, we would typically choose a hybrid silica column, like YMC-Triart hybrids, over nonhybrid options due to the expectation of increased reproducibility and robustness.”



YMC-Triart C18 columns exhibit lot-to-lot reproducibility for all types of compounds, including basic and coordination compounds that often give rise to peak tailing or adsorption onto packing material.

Image credit: YMC America, Inc.

CONCLUSION

Optimized separation processes are crucial for maintaining the efficiency of drug development workflows. To minimize bottlenecks in analytical separation and preparative purification, pharmaceutical scientists must take special care to use the most suitable HPLC columns. As such, the industry is now beginning to branch out from traditional silica support materials to more customized hybrid

products that offer superior resolution, reproducibility, and robustness. Hybrid silica products have seen quite a few developments over the past two decades to improve these qualities.

“One further advancement I’d like to see in the hybrid industry would be a superficially porous particle hybrid,” Dong says. Superficially porous particle (SPP) hybrids consist of a solid, nonporous silica core surrounded by a porous shell.¹² The marriage of a solid core with a porous shell gives rise to better column efficiency in HPLC separation. Dong thinks an SPP-hybrid particle would increase efficiency even further.

Recently, biopharmaceuticals such as peptides, proteins, oligonucleotides, and antibodies have become popular targets for purification. Naturally, new demand for analysis of these agents will arise. According to Sato, “YMC is currently focusing on the biopharmaceutical market, and on developing customized hybrids that can be used for both analytical and preparative separation of new types of biotherapeutics.”

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