A Q&A

Transferring Analytical Methods: Smoothing the Transition

Mike Churchill
Product Manager
Develosil

Making the transfer between HPLC and UHPLC more predictable.

From faster throughput to cost efficiency, laboratories have plenty of reasons to transfer analytical methods from conventional high-performance liquid chromatography (HPLC) to ultra-high-performance liquid chromatography (UHPLC).

However, the benefits of this transition also come with costs, including the time and labor involved in validating the new methods. Perhaps even more vexing is the unpredictable effect that the transfer between HPLC and UHPLC can have on separation selectivity. Fortunately, solutions for this issue exist, and given the advantages of UHPLC, such solutions are worth pursuing.

LCGC sat down with Mike Churchill, product manager at Develosil, to learn about the available options and how working with a single line of columns can make the entire process more efficient and predictable.

LCGC: Why do laboratories need to transfer methods between HPLC and UHPLC?

Churchill: There are several common scenarios. An R&D laboratory in an organization may have updated to UHPLC to increase throughput and decrease solvent usage during discovery; meanwhile, in the quality control (QC) laboratory, those benefits may not have outweighed the costs of acquiring a second platform and validating new methods yet.

As a result, methods developed for R&D for new products may need to be 'back transferred' to HPLC before the QC laboratory can begin validating them. This also frequently happens when a process is scaled up from an initial analytical UHPLC method to semi-preparative or preparative HPLC methods.

In other cases, QC laboratories may wish to update their legacy methods to UHPLC after buying new equipment. This lets them run everything on a single platform and maybe retire their legacy equipment.

We have found that the process is more efficient and predictable if the same line of columns with the same underlying performance can be used throughout, no matter if the transfer happens between different departments in an organization or even just a single user.

LCGC: What is the biggest challenge when transferring methods between HPLC and UHPLC?

Churchill: The biggest challenge is often dealing with unanticipated changes in selectivity when changing to a different particle size for the separation column.

The advantages of UHPLC are dependent on scaling down both the particle size and column dimensions from those used in an HPLC separation. There are many software packages provided by chromatography vendors, as well as *LCGC*/CHROMacademy, that allow analytical chemists to calculate those solutions for a method transfer. These programs also handle changes in particle size. As long as the instrument parameters, column parameters, injection, etc., are input correctly and rules are followed, geometrical scaling rules should apply and the software should accurately solve for the gradient, the flow, and the column size for the new method.

The predication fails and the challenge often begins when scaling the method to or from UHPLC requires a change in particle size. HPLC is typically restricted to 3–5-µm particles while UHPLC takes advantage of the high efficiency of 1.6-µm particles.

This challenge would be easy to deal with if

SPONSORED BY

Develosil®

LC|GC north america

the only changes were the size of the particle while everything else stayed the same. However, different particle sizes—even within a single product line—may actually display changes in selectivity. This is often because manufacturers have to change chemical-bonding methods, or even the silica base material itself, when working with different particle sizes and different pressure ratings.

The large particles suitable for HPLC may not be suitable for high-pressure use, so the small particle size may start with a different silica or a different bonding process for endcapping or functional groups. The different particle sizes can end up with different porosity, carbon loading, differing amounts of uncapped silanol, and other properties; all of these can add up to a change in selectivity.

LCGC: How can this process be made more predictable?

Churchill: *LCGC*'s CHROMacademy tutorial and other articles are great references for measurable properties of a packing material that affect selectivity. With enough data about each individual column and additional factors in your model, the predictions can be made more accurate. Nevertheless, it is a lot easier to work with columns that are manufactured to be fully scalable from UHPLC up to HPLC.

Develosil's Flexfire columns are made so that the silica gel, endcapping, and bonding of functional groups are extremely homogenous. The columns also provide predictable consistency across all the different Flexfire columns; only the particle size changes.

Flexfire displays the same surface area ratio of 340m²/g, pore size and dispersion, bonding/loading of functional groups, and highly complete endcapping on 1.6-µm particles as well as on 2.6- and 5-µm particles.

The dispersity of the size of the particles is also tightly controlled, which is critical when trying to maintain a high efficiency and predictable separation.

To reiterate, as long as rules for factors such as injection volume and extra column volume are respected, highly predictable method transfers will occur.

LCGC: In addition to changes in packing material and scale, there might also be changes in detection when the method is transferred. Are these columns and methods compatible across the range of detectors (e.g., ultraviolet–visible spectroscopy [UV–Vi], refractive index, mass spectrometry [MS]) available today?

Churchill: The columns themselves are completely compatible with all the major detection methods, and laboratories can plan ahead so that their methods can easily transfer between different detectors. Even if UV–Vis is used in one laboratory, MS might be used in another.

Choosing only volatile buffers, pH modifiers, and ion-pairing agents is necessary for liquid chromatography–mass spectrometry (LC–MS) applications but choosing these ahead of time for UV–Vis applications when possible allows for flexibility in the future (e.g., the transfer of UV–Vis methods to LC–MS methods).

This approach is also useful for scale up. For instance, using a phosphate buffer involves having to scale up for purification later on in the process, which leads to a desalting step. However, using fully volatile buffers could allow for that extra step to be skipped.

Similarly, using acetonitrile as the organic modifier across all your methods can be helpful because the acetonitrile can increase ionization efficiency for MS detection, increase UV transparency for UV detection, and decrease backpressure on all your methods.

LCGC: Switching to a new line of columns can create significant paperwork and validation studies for GMP and QC laboratories in addition to the time spent optimizing the actual conditions. Switching legacy applications from HPLC to UHPLC also presents a large investment in validating the updated methods. How can Develosil Flexfire columns help justify the expense?

Churchill: While there may be many upfront costs, the increased durability and predictability of Flexfire columns means less time spent in the long run. The low dispersity in both pore size and particle size as well as improved endcapping keep runs reproducible for a longer period of time.

Develosil's manufacturing process not only makes Flexfire columns more predictable, but it also makes columns significantly more robust. Improved endcapping technology means Flexfire columns are stable up to pH 10, which enables them to operate under alkaline conditions; at neutral pH, the columns last remarkably longer than other columns.

Part of notion that UHPLC columns and hardware need to be replaced more frequently is created by runtime (e.g., a thousand injections go a lot faster on UHPLC than on HPLC), so even though the productivity is the same, the user has gone through columns a bit faster.

LCGC: What other factors need to be taken into account when transferring methods to UHPLC?

Churchill: There are many factors specific to the individual HPLC and UHPLC systems themselves, such as each system's dwell volume and extra column volume—also known as the system dispersion.

Small columns have correspondingly finer frits, which are easier to clog than HPLC column frits. For UHPLC, 0.2-µm filtering of samples and mobile phases is critical to prolonging the life of UHPLC columns.

Also, before you install a new column on your HPLC or UHPLC, always confirm that fittings and ferrules are correct and properly spaced. Ferrules at the wrong distance from the end of the tubing can create a void, which will add a dead volume or a leak. This is one more reason to stick with a single line of columns if possible—to ensure that the ferrule is always at the correct position.