14 Years on from the Introduction of UHPLC

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This article describes the growing pains associated with the implementation of UHPLC (Ultra High-Performance Liquid Chromatography) in pharmaceutical laboratories, its maturity as a major chromatographic technique and possible future trends for its continued growth and expansion.

Introduction

'Tempus fugit': it only seems like yesterday since the first commercially available LC instrumentation capable of operating in the region of 1000 bar (termed ultra-high-performance liquid chromatography [UHPLC]) was introduced. Fourteen years later, UHPLC has been successfully and widely established as the chromatographic technique of choice in many analytical disciplines ranging from research / academic environments to quality control/operations functions [1, 2].

From Child Diseases to the Fast Track of Instrument Launches

Time is a great healer, implementing UHPLC in the pharmaceutical industry in the early days was not an easy task, users were plagued with numerous reliability issues. However, by working closely with visionary end-users, UHPLC instrument and column manufacturers were able to identify and resolve these practical issues. This type of close collaboration between instrument and column designers, engineers and end-users is a highly attractive development model that is now widely used to fast track new instrumentation to the marketplace [3, 4, 5].

Many chromatographers instigated a slow strategic stepwise implementation of UHPLC, initially starting with experts and finishing up with general users. This process successfully demonstrated the expected advantages of this technique, namely improvements in data quality (i.e. enhanced efficiency leading to improved resolution) and increased speed, hence the ability to analyse larger sample numbers as well as labile analytes.

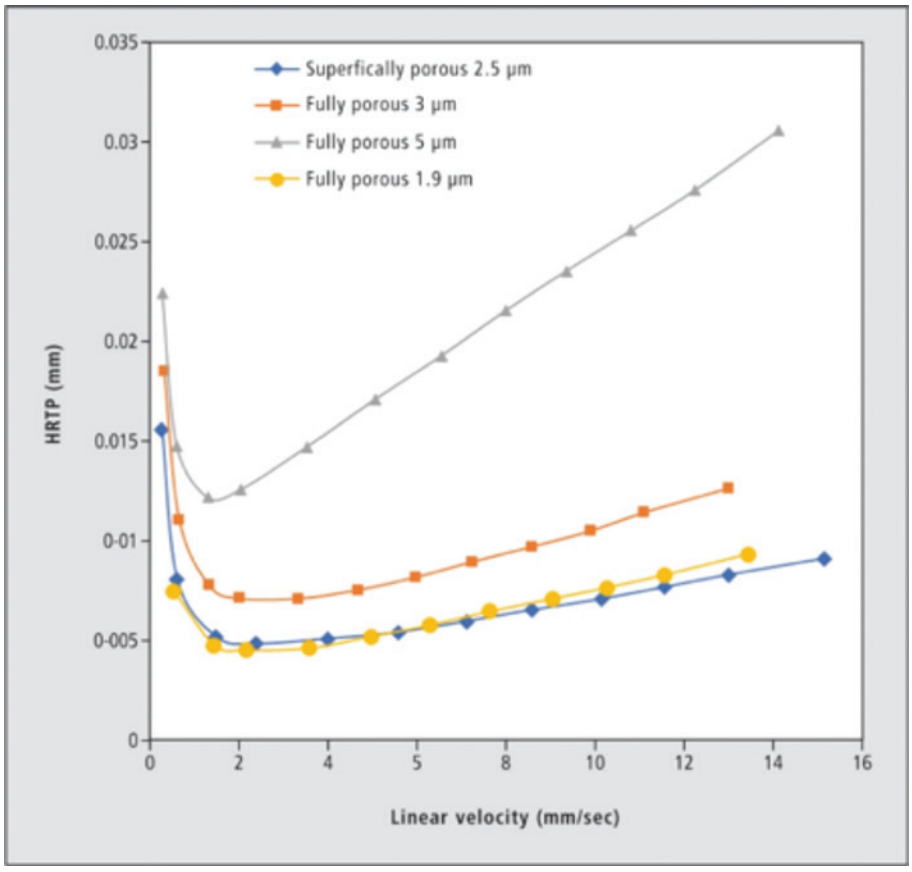

Figure 1. Van Deemter Plot of plate height versus mobile phase linear velocity for a variety of different particle sizes.

Higher Productivity at Lower Cost

As a direct consequence of the increased productivity, it has been shown that it is feasible to replace approximately two to three HPLC systems with one UHPLC while maintaining the same, or better productivity at lower cost [6]. A reduction in LC-system numbers also has the benefit of reduced instrument qualification, maintenance and repair costs. The ability to obtain results on the same day by increasing speed of analysis is important for process control, cleaning validations and to ensure reliable results, by checking for System Suitability Test (SST) failures before the run is commenced. The

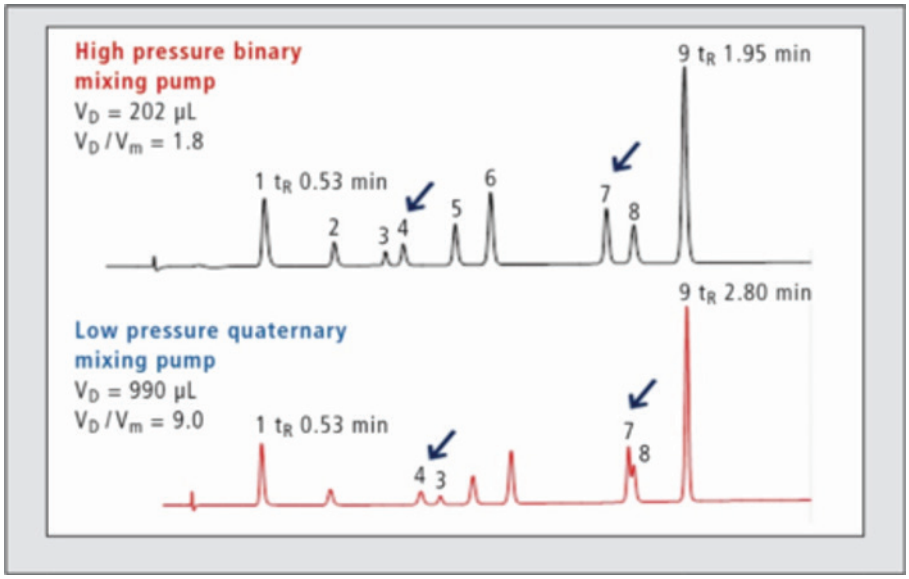

Figure 2. Change in gradient selectivity due to differences of delay volume in a high pressure and low pressure gradient system [7].

capability to run a large number of samples and to pool and queue samples to be run using a generic 'walk-up' UHPLC system is extremely attractive in high throughput laboratories.

In theory, the use of smaller internal diameter columns (2.1 mm or 3 mm) should result in reduced solvent consumption but, in practice, the gains are not as great as predicted [7]. However, one of the biggest drivers for using UHPLC in a quality control environment is the fact that the increased speed of analysis facilitates quicker release of commercial products to the market.

UHPLC 1000+ Bar Systems

UHPLC systems capable of operating at high pressures were developed primarily for high linear velocity chromatograph columns which were packed with fully porous sub two-micron particles (1.7 µm - 1.9 µm). These small particles have been demonstrated to generate extremely favourable van Deemter and kinetic plots (Figure 1), that is, maintaining high efficiencies even at high flow rates [8, 9]. This necessitated LC instrument components (i.e. pistons, seals, tubing, flow cells etc.) able to withstand prolonged operation at pressures of up to 1000 bar.

Due to the narrow peak volumes and resulting sharp peaks generated with columns packed with sub two-micron particles, it was imperative that UHPLC instrumentation had a substantially lower system dispersion volume (approx. 15 µl) compared to that of standard HPLC systems (approx. 55 µl), in order to avoid peak dispersion and to maintain these narrow peak widths [10]. Coupled to this, the detector data acquisition rate had to be increased to provide sufficient data points over these narrow peaks in order to describe them adequately.

Most instrument manufacturers now offer UHPLC systems with maximal operating pressure of 1000 - 1500 bar. Interestingly though, most UHPLC columns are only capable of being operated at up to 1000 bar! UHPLC columns must be properly installed so that no extra dead volume is introduced and care should be taken, that the column fittings and tubing do not slip during use, while exposed to elevated pressures, resulting in unwanted system dispersion volume.

The first types of UHPLC systems were based on a binary pump configuration with only one column position, limited detector choice and a fixed loop injector autosampler. Nowadays, there is a choice between either quaternary (low-pressure mixing) or binary pumps (high-pressure mixing). If increased flexibility of multiple solvent blending is needed, as often used in method development strategies, then a quaternary pump should be selected, but this brings about an inherent increase in system dwell volume compared to a binary pump configuration. Care must be taken to account for these differences in system dwell volume when transferring a method from one system to the other, as variation in dwell volume can have adverse effects on gradient selectivity (Figure 2).

To overcome these differences in system volume most software now offers a timed injection feature. If the gradient run is started before sample injection, gradient delay will be reduced by the time the injection is delayed (Figure 3).

Column ovens now offer the inclusion of column switching valves, as an option for chromatographers to evaluate up to six different columns with differing stationary phase chemistries and selectivities. With a view to reducing pressure and allowing even longer columns to be used temperatures can be programmed to 80°C or higher.

The extra-column dispersion of UHPLC instrumentation has been substantially reduced compared to that of standard HPLC systems, again to maintain the narrow peak volumes and widths. A wide range of detectors is now commercially available for UHPLC systems, ranging from variable UV, diode array, fluorescence, refractive index,

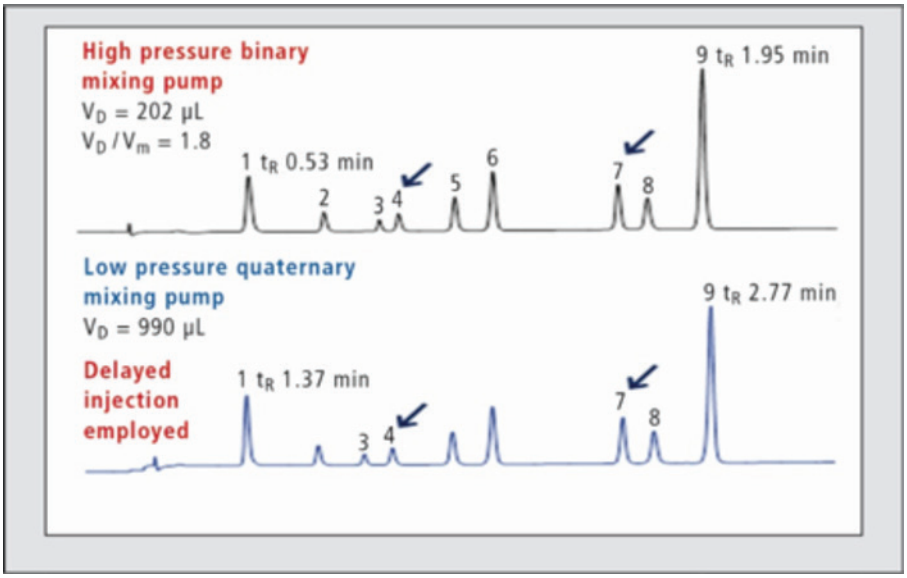

Figure 3. Use of delayed sample injection to account for differences in delay volume of high and low pressure gradient system [7].

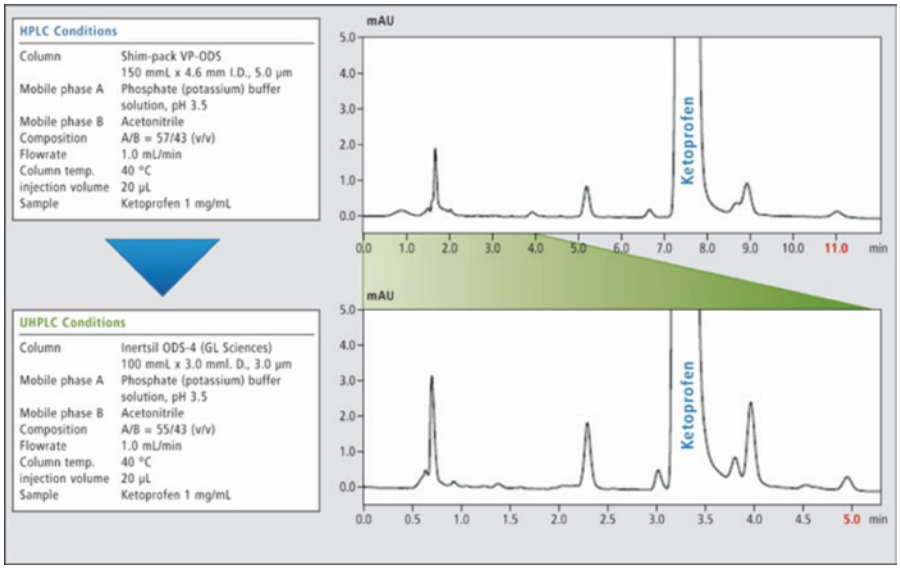

Figure 4. Method transfer of a 11 min HPLC assay of Ketoprofen and related substances to the equivalent 5 min UHPLC method by using a method transfer calculator to maintain selectivity.

evaporative light scattering, corona aerosol, electrochemical and a variety of easy to use mass spectrophotometric detectors. Due to the low peak volumes, low volume flow cells have had to be designed, many possessing extended path lengths to maintain good signal to noise ratios.

As a consequence of the rapid analyses that are now possible with UHPLC, the speed of autosampler injection, and associated wash sequence, has had to be increased considerably. Many autosamplers can now draw a sample and fill the loop or holding capillary while the previous sample is still being run. Originally, fixed loop autosamplers were introduced to minimise the dwell and system volume of the UHPLC system. However, despite variable injection volume autosampler UHPLC systems having a much larger dwell volume, their convenience and flexibility compared to loop autosamplers has resulted in most of the major instrument manufacturers now offering them.

UHPLC 660 Bar Systems

The introduction of 2.7 µm superficially porous materials, suitable for HPLC as well as UHPLC analysis, in 2007, and the explosion in their use as a result of their ability to generate similar efficiencies to sub two-micron materials at pressures just slightly higher than typical 3-micron packed columns when run on UHPLC systems (see Figure 1), has resulted in a new generation of UHPLC systems with a slightly lower maximal pressure capability of up to 660 bar.

Practical UHPLC

Reliability of current UHPLC instrumentation is excellent as long as the operators have been suitably trained. Smaller tubing and filter frits on UHPLC columns are prone to clogging, so crude samples have to be filtered and good quality water, salts and modifiers need to be used. Flushing buffered solvents from the system to avoid precipitation should be routine practice after finishing an analysis.

The largest issue associated with UHPLC is microbial contamination of the mobile phase which can cause blockage of the typical 0.2 µm column frits over time, i.e. smaller than most bacteria. This results in a build-up of bacteria on the inlet frit and a gradual increase in the back pressure during the lifetime of the column.

We have previously shown that the bacteria responsible for contamination of mobile phase reservoirs were mostly of human origin (i.e. from the operator during the mobile phase preparation) [11], hence it was recommended that good quality salts and additives be used, water be freshly dispensed from a water purification unit, UHPLC grade organic modifiers are used and that as little human manipulation as possible is performed (i.e. no filtering if possible). It is recommended that filter valves be used on the mobile phase reservoirs as these prevent ingress of bacteria into the mobile phase from the environment and also prevent evaporation of the organic constituent.

End-user installed in-line filters to remove any fibres and particulate debris from pump seals preventing column and UHPLC tubing blockage are not normally required with the new generation of UHPLC systems. It is good practice for HPLC and UHPLC to consider the particulate load of the sample that is being injected - standard practice is to filter or centrifuge samples which have particulate matter in them, however, for most pharmaceutical work this has not been necessary - so the chromatographer must assess this on a case by case basis.

Initially, many UHPLC columns were introduced that contained very fine particles (sub 1 micron), which resulted in blockage of the column frits and excessive back pressure as a function of column use, yielding unusable columns. These problems were soon rectified and a wide range of extremely robust and reliable UHPLC columns are now available which can be used up to 1000 bar.

Similar detection limits should be achievable to those seen with HPLC assuming that the injection volume has been scaled accordingly, the correct flow cell has been selected and that there is no excessive system dispersion associated with the UHPLC system.

Retention modelling is now ubiquitously used within chromatographic method development strategies [12], and it has been reported recently that retention modelling and optimisation using UHPLC generated input data yields far more accurate predictions than previously observed with older HPLC systems. This was partly attributed to more accurate gradient formations with these new generation UHPLC systems and their greater reliability [12].

Translations Between Differing UHPLC and HPLC Systems

The disparity in dwell volume between UHPLC systems and HPLC systems can be vast. Certain UHPLC configurations have a stated dwell volume of 100 µl compared to HPLC values which are typically in excess of 1000 µl. This can have major repercussions when performing method transfers between HPLC and UHPLC and vice versa. For example, differences in selectivity can often be observed which result in a failed system suitability test (SST).

Most instrument manufacturers now offer method translation tools and also independent software programs are available (e.g. Translate My Method - ACD [13]). These can be used to assist in the translation of isocratic and gradient HPLC to UHPLC conditions, as shown in an example in Figure 4.

However, method translation software cannot always take into account that pressure can have a profound effect on retention and hence selectivity of certain analytes [7, 14]. In this case, it is recommended that the end-user performs retention modelling (i.e. from two or three input chromatograms) using the new chromatographic system with the new pressures and then re-optimises and confirms that the new conditions will still pass the SST at the new pressure conditions.

Future of UHPLC

UHPLC is now firmly embedded within most chromatographic environments, ranging from research and academic environments to quality control and operations functions, and its use and applicability will no doubt increase. It is quite possible that we will see newer generations of UHPLC systems capable of delivering higher pressures to drive even smaller totally porous or superficially porous particles sizes or pillar array columns. This may impose a re-design of instrumentation to cope with the requirements of even smaller system dispersion and dwell volumes, which could be achieved through chip technology. Smart software to drive more sophisticated method development strategies will be essential.

At present, current UHPLC is not really compatible with column internal diameters of 1 mm - hence it is expected that UHPLC instrumentation capable of using capillary and nano column formats will be developed in the near future. If this is true, then major advances in detector sensitivity (i.e. for non-MS detectors) will be needed. Obviously, the introduction of any new technology must be considered holistically, as failure to re-design or optimise all the UHPLC components (i.e. failure to correspondingly reduce system volume) may result in sub-standard performance.

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