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Use of ultra-performance liquid chromatography in pharmaceutical development

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Abstract

Ultra-performance liquid chromatography (UPLC) has been investigated as an alternative to HPLC for the analysis of pharmaceutical development compounds. We present data on three compounds showing that significant reductions in separation time can be achieved without compromising the separation quality. Results from precision and comparative studies indicate that UPLC is a suitable technique for routine pharmaceutical analysis.

Keywords: UPLC; Ultra performance liquid chromatography; Pharmaceutical development

1. Introduction

1.1. The role of HPLC in pharmaceutical development

Pharmaceutical development is an important part of the phase in between the identification of a chemical entity with therapeutic potential and the launch and routine use of a new medicine. A number of activities are involved including the scale up of the synthetic route from bench to plant scale, and the development of a tablet or other dosage form of the new medicine which can be manufactured at a large scale. Analytical chemistry plays an important role in supporting these activities by helping to understand the impact of changes in the route and scale of manufacture on the quality and consistency of the dosage form.

HPLC is one of the main analytical techniques used in controlling the quality and consistency of both the chemical entity, or active substance, and the dosage form. For example, HPLC is used in determining the purity of different batches of the chemical entity and so helps to ensure that material used in clinical trials is of a similar quality to that which has been assessed in toxicological studies. HPLC is also used to determine whether any degradation of the chemical occurs within the dosage form over time and so helps to establish the shelf life.

1.2. Improving efficiency in HPLC

Whilst HPLC is a very well established reliable technique, and is adequate in controlling pharmaceutical quality and consistency, it could still be improved. For example, one problem is that HPLC is often a slow technique because of the complexity of some of the pharmaceutical samples encountered. For example, samples may contain several impurities at levels of around 0.1% relative to the active substance. Such a concentration range means that the separating column must have high sample capacity in addition to high efficiency. These low level impurities can include species such as residual intermediates, analogues of the active substance, isomers, and degradation products. These must be separated sufficiently from the active substance and from each other so that their concentrations can be reliably measured. Because of the range of polarities involved gradient separation are often required for purity assessment, and because of sample complexity separation times of 30 min or more are not uncommon. Reducing these separation times without reducing the quality of the separation would mean that important analytical information could be generated more quickly.

Reducing separation times in HPLC without reducing the quality of the separation requires generating higher resolving power per unit time. Whilst the resolution between individual analytes in a particular sample may be increased by improving selectivity or retention, the best general approach to increasing resolving power is to increase separating efficiency.

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In this paper the use of a smaller diameter packing and higher operating pressures has been explored as a way of generating higher separating efficiencies. A commercial system capable of generating much higher pressures (1000 bar) than used in standard HPLC has been evaluated to determine its potential in routine analysis.

1.3. The need for higher pressures

Efficiency in HPLC is a function of operating conditions. Extensive experience has shown that the height of the column equivalent to a theoretical plate (HETP) varies with the mobile phase linear velocity. Efficiency in packed column HPLC can be described by the plate height model used in the van Deemter equation, and other equations of a similar form. In this work, Eq. (1) has been used as it makes explicit the impact of column properties, analyte properties and operating conditions on column efficiency [1].

$$H = Ad_p + \frac{BD_M}{u} + \frac{Cd_p^2 u}{D_M} \quad (1)$$

where H is the HETP, d_p the particle size of the column packing material, u the linear velocity of the mobile phase, D_M the analyte diffusion coefficient and A–C are the constants. A relates to the fact that the flow path through a packed bed is tortuous and so not all analyte molecules have paths through the column of equal length. B relates to molecular diffusion (in the direction of the column axis). C describes the mass transfer of the analyte in the mobile and stationary phases. [1].

Other equations of greater complexity than 1 can be employed depending on the purpose. For a fuller analysis, description, and discussion of the underlying physical processes that underpin chromatography the reader is referred to more specialised texts [1,2].

Eq. (1) shows that efficiency varies with linear velocity, and the nature of the second and third terms of the equation indicates a minimum value for HETP. This minimum occurs at linear velocities that are much lower than those typically employed with stationary phase particles in the range of 5–3 μm in diameter. In the third term of Eq. (1), the particle size is squared and so the curve is steeper for larger particles at high linear velocities. This means that in order to reduce analysis times to acceptable values columns packed with common particle sizes are often operated at linear velocities which do not give maximum efficiency.

The position of the minimum on the HETP curve, and the optimum linear velocity, can be determined by the use of differential calculus [2]. The optimum linear velocity occurs when the slope of the H versus u curve is zero, i.e. when $dH/du = 0$. This condition is satisfied when:

$$u_{opt} = \frac{D_M}{d_p}\sqrt{\frac{B}{C}} \quad (2)$$

Eq. (2) shows that the optimum linear velocity is inversely related to the particle size, and directly proportionate to the analyte diffusion coefficient. Optimum operating conditions depend upon the analyte as well as the column employed. The use of smaller diameter particles should allow the use of higher linear velocities and so shorter analysis times. Smaller analytes (larger diffusion coefficients) can be analysed at higher linear velocities.

The value of H at the optimum linear velocity can be obtained by substituting the value of u given in Eq. (2) into Eq. (1).

$$H_{min} = d_p(A + \sqrt{BC}) \quad (3)$$

So as long as the same values of A–C can be obtained the minimum value of HETP is directly proportional to particle diameter.

Eqs. (1) and (2) can be used as the basis for determining which approaches may be beneficial in improving operating speed and efficiency in HPLC. From the discussion above it can be seen that benefits could be gained by reducing the size of the stationary phase packings that are employed in pharmaceutical analysis. Smaller particles give the potential for columns with higher optimum linear velocities and at the same time columns whose efficiency is less dependent upon linear velocity.

The main difficulty with using smaller diameter packings is that the pressure required to pump the mobile phase through the column increases with the square of the particle diameter [3]. In order to gain the full benefits of small particles higher operating pressures are required than can be obtained with standard commercial systems.

1.4. Ultra-high-pressure liquid chromatography (UHPLC) and ultra-performance liquid chromatography (UPLC)

1.4.1. UHPLC

Whilst commercial HPLC systems typically have a maximum operating pressure of around 400 bar there has been interest for a number of years in the benefits of higher pressures. Work in academic laboratories has employed non-porous silica and zirconia based packing materials with diameters in the range of 1–1.5 μm, and used pressures which are one order of magnitude greater than those found in HPLC [4–9]. Non-porous particles are used because of their mechanical strength and relative ease of manufacture. In addition, fused silica capillaries with diameters in the range 10–150 μm are used to minimise the impact of frictional heating. In general, higher efficiencies are obtained from narrower capillaries [8,10] with systematic changes to the A and C terms of the van Deemter equation being observed [10]. Because of the very high pressures involved special equipment is required to pump the mobile phase and to pack the columns. The term UHPLC has been used to distinguish this technique from conventional HPLC. The use of UHPLC has given efficiencies of up to 300,000 plates per column for analytes such as hydroquinone [4]. Pharmaceuticals such as benzodiazepines were separated and detected by either UV absorbance or time of flight MS [7]. Gradient UHPLC was also employed to separate the many peptides produced by a tryptic digest of ovalbumin [5].

In pharmaceutical development, the large sample concentration ranges inherent in purity assessment (see above) mean that porous packing materials are normally employed in HPLC. Porous packing materials capable of withstanding higher pressures are now available and they have been shown to give much