UHPLC: Matching Filtration Technology to Particle Size

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When dealing with analytes in complex matrices, extensive sample pre-treatment may be carried out in order to produce a 'clean' sample. However, in the interests of speed, compromises may often be made. In such cases, careful consideration must be given to filtering sample solutions (incidentally also to avoiding precipitation caused by solubility differences between sample solvent and mobile phase). With the ever-increasing use of UHPLC with expensive reduced dimension columns and packing materials, greater attention needs to be paid to sample filtration issues.

Selection of filter pore size for liquid chromatography applications

Of the four common causes for high performance liquid chromatography (HPLC) column failure (plugging, column voiding, sample adsorbtion, and instability of the stationary phase to chemical degradation), plugging is the most frequently encountered by analysts. Injection of samples containing particulates will eventually block the column inlet, cause high column back-pressure, peak deformation and shorten the normal lifetime of the column. Operations of pump components, injectors, and detectors are expected to be less troublesome when fluids are filtered.

The choice of filter pore size for optimal filtration should be determined based on the column packing size. Ideally, an analyst would not want contaminated particulates to fit into the space between the particles of the packing. This space (labeled 'Flow Path') is identified in Figure 1. The premise for filter pore size selection is to find out how large the flow path space is in order to understand what size of particles require to be removed from the mobile phase. For example, starting with a 3 µm packing size and drawing concentric equilateral triangles (Figure 2), the largest particle capable of fitting through the column by circumscribing an equilateral triangle with a side length of 0.75 µm can be determined (assuming a perfect (cubic close packing) arrangement).

Figure 3 illustrates a right triangle whose short side describes the radius of the sphere shown in Figure 2. The angle is one half of 60°, or 30°. The horizontal side of the new right angled triangle has a length of 0.375 µm (half of 0.75 µm). Calculating the tangent of 30° gives the ratio of the length of the opposite side over the adjacent side, in this case 0.58. Therefore the shortest side of the triangle is equal to 0.58 x 0.375 or 0.217 µm in length. Coincidentally, this is also the radius of the particle. So, if the column packing is 3 µm in diameter, the flow path is 0.43 µm. In general the following equation applies to determining the flow path radius;

$$Flow\ Path\ Radius = \frac{d_p}{8} \tan 30°$$

where dp is the particle diameter of the packing material in the column.

Therefore, when an HPLC column has a packing size of 3 µm or smaller, a 0.2 µm filter should be used because a 0.45 µm filter may let particles through that will plug the column. For liquid chromatography systems using columns with packings larger than 3 µm, the filtration industry standard of 0.45 µm for syringe filters and mobile phase membranes is sufficient.

However, for columns with 3 µm or smaller packings, including UHPLC, a 0.2 µm filter is recommended. Also, in selecting a suitable filter it is also important to consider the material used.

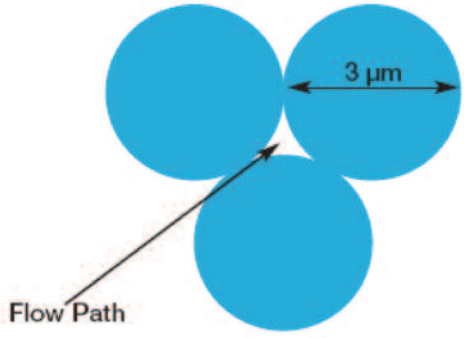

Figure 1: Flow path formed by spherical particle packing

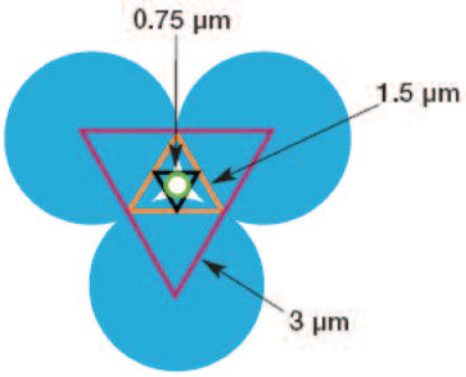

Figure 2: Initial determination of flow path dimensions using a concentric triangle approach

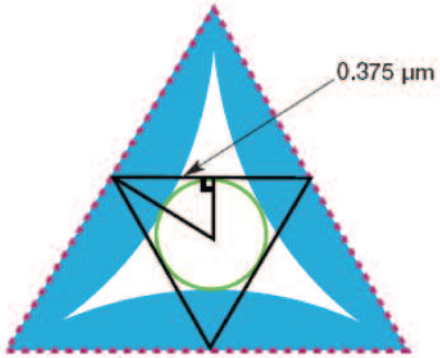

Figure 3: Initial determination of flow path dimensions using a concentric triangle approach

It is very important that this must be matched to the types of sample being analysed in order to reduce analyte binding and maintain accuracy of methods. Typical materials used in modern filters include GHP (polypropylene), PVDF, Nylon, and PTFE membranes providing a wide range of solvent compatibilities. GHP hydrophilic polypropylene (GH Polypro) is ideal for chemical compatibility, fast flow rates, low protein binding, and low levels of UV-absorbing extractables.

Glass fiber prefilters allow for quick and easy filtration for difficult-to-filter aqueous solutions.

PVDF membranes have excellent compatibility with aggressive acids and alcohols. PES (hydrophilic polyethersulfone) membranes can be found with special ion chromatography designs or with GxF prefilters. Hydrophilic nylon membranes have excellent compatibility with esters, bases, and alcohols, and require no prewetting. Hydrophobic PTFE membranes with polypropylene support offer good chemical and temperature compatibility and are ideal for gas, organic solutions, air filtration, or venting. Potential extractables are also reduced through use of a polypropylene housing for the filter.

Other significant design features that should be taken into account in good filter design are good flow characteristics, lack of UV absorbing extractables, low hold-up volumes and compatability with robotic systems.

Returning to the key issue of filter pore size, a short study was carried out to confirm the anticipated superior protective properties of a 0.2 µm Acrodisc MS syringe filter relative to a 0.45 µm rated filter for use with UPLC columns.,

A dilute 0.05% (w/v) suspension of 0.31 µm (average diameter) polymer microspheres were repeatedly injected onto a UHPLC column. The size of this particle is representative of those that might be found in a bioanalytical assay where no sample preparation has been performed.

Therefore, while this represented a much simpler system than would be found in, for example, a protein crash from a biological extract, it served to address the issue of filter pore size. An unfiltered suspension was used as a reference control. The lifespan of the UHPLC column was deduced by monitoring column backpressure.

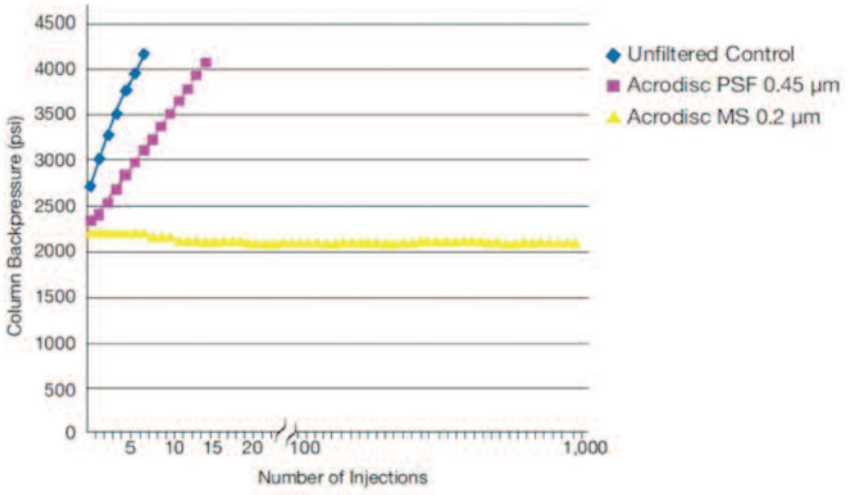

Figure 4. Impact of filtration on UHPLC column pressure with repeated injection of a 0.31 µm (average diameter) polymer microsphere solution

Materials and Methods

- Waters HPLC system with 1525 binary pump and autosampler model 2707
- Acquity UPLC BEH C18 column, 50 mm x 2.1 mm, 1.7 µm particle size (Waters Corp., PN 186002350)
- Pall 0.2 µm Acrodisc MS syringe filters (PN MS-3201)
- Pall 0.45 µm Acrodisc PSF syringe filters with nylon membrane (PN AP-4502)
- Polymer microsphere, 0.31 µm, 10% w/w suspension (Thermo Scientific, PN 5031A)
- Triton X-100 (Sigma-Aldrich, PN 21568)
- Acetonitrile, BAKER ANALYZED HPLC ultra gradient solvent (J.T. Baker, PN 9017)
- Water ChromAR, HPLC-grade (Macron Fine Chemicals, PN 6795).

The mobile phase was water - acetonitrile (33:67, v/v) and the flow rate was set at 0.3 mL/min in order to work within the pump operating pressure range. The injection volume was 8 µL, and the column temperature was maintained at 35 °C (95 °F).

Procedure

A 0.05% microsphere suspension (average diameter 0.31 µm) in 0.002% Triton X-100 (prepared from a 10% w/w polymer stock), was filtered using either 0.2 µm Acrodisc MS syringe filters or 0.45 µm Acrodisc PSF syringe filters with a nylon membrane. Effluents were collected in 1.5 mL aliquots and 20 replicate effluent samples were injected onto the UPLC column. The unfiltered material was used as a negative control. The initial UPLC column backpressure was approximately 2,000 psi for all three samples (unfiltered control and effluents from the 0.2 µm Acrodisc MS syringe filters and 0.45 µm Acrodisc PSF syringe filters with nylon membrane). The analysis was stopped when the back pressure from the UPLC column reached approximately 4,000 psi.

Results and Discussion

Injection of the unfiltered and the 0.45 µm filtered suspensions resulted in a rapid and significant increase in the backpressure on the Acquity UPLC column, after 9 and 16 injections respectively. By contrast, column plugging was not observed even after 1,000 injections of the effluents from the 0.2 µm filters. A summary of the results is shown in Figure 4.

Conclusions

Clearly 0.45 µm syringe filters are unsuitable for filtering samples for UHPLC analysis. The results of the study carried out demonstrated the effectiveness of the 0.2 µm filters at extending UHPLC column life. Based on measurements of column backpressure following repeated sample injections, filtration using these filters prolong the lifespan of the UHPLC column at least 50-fold relative to filtration using 0.45 µm syringe filters and over 100 fold compared to use of unfiltered samples. The cost-savings generated by this simple filtration step are likely to be very significant.