Syringe Filter Efficiency and the Effect of Filtration on HPLC Column Life

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High performance liquid chromatography (HPLC) is the most common analytical technique in the biopharmaceutical industry. Particulates in HPLC columns result in increased column back pressure, disrupted nominal band shape and reduced plate number, consequently shortening column life and making analytical results difficult to interpret. Using appropriate sample filtration prior to injection is an efficient, cost-effective way to remove particulates and extend the life of HPLC system components. In this study, various syringe filters from different manufacturers are quantitatively evaluated for column protection ability. The average retention efficiency of the 0.45µm rated syringe filters from three different suppliers at removing 0.45µm average diameter latex spheres, ranges from 95% to 33%. The best filter significantly prolonged HPLC column life compared to the worst filter allowing at least 26 times the number of injections, with no significant increase in column back pressure, thus demonstrating that sample filtration prior to injection can and does significantly extend the life of HPLC columns. The mechanism of column plugging is also discussed.

Of the four common causes for high performance liquid chromatography column failure – plugging, voids, absorbed sample and chemical attack – plugging is the most frequently encountered by analytical chemists or analysts. Injection of samples containing particulates will eventually block the column inlet and column packing, cause high column back-pressure and shorten the normal lifetime of the column (1). Operations of pump components, injectors and detectors can be expected to be less troublesome when fluids are filtered. For HPLC...
applications, the 0.45µm pore size filter is typically selected for removal of particulates (2). Although there are several seemingly equivalent products on the market, lack of knowledge about the differences between filters leads to more frequent column replacement and extensive operation downtime.

Filtration as a preventative maintenance tool for HPLC analysis is well-documented (3-5). It is commonly taken for granted that samples are filtered prior to injection, but the extension of the column life has not been well quantified. It is the intent of this work to demonstrate that filter efficiency must be considered when choosing an HPLC sample-prep filter and that filtration will lengthen the life of a column. In this article, retention efficiency of three apparently equivalent 0.45µm rated syringe filters from three different filter vendors was examined using 0.45µm average diameter latex spheres. This work was conducted with latex spheres to offer the best possible reproducibility in both sample preparation and filter efficiency measurements. In order to correlate the retention of spheres with the actual application, the quantitative effect of filtration on HPLC column life was investigated. This involved examining column life without filtration, as compared to column life when samples were filtered. It should be recognised that extending the column life is dependent on the particulate within the sample and actual column life extension may vary.

**TESTING**

The UV/VIS spectrophotometer was used for measuring absorbance of latex-sphere solutions. The maximum absorbance of the latex-sphere solution was observed at 272nm, which was used to correlate latex sphere concentrations with absorbance. The surfactant solution, 0.1% Triton X-100 that is free of latex spheres, was measured as the blank at 272nm. A series of standard solutions of 0.0025%, 0.0050%, 0.0075% and 0.01% 0.45µm latex sphere concentrations were made and used for creating the calibration curve. The linear relationship between latex sphere concentrations and absorbance was established, which is in accordance with Beer’s law (2). A correlation coefficient of 0.9999 was obtained. A 0.01% 0.45µm average diameter latex sphere solution was used as the challenge solution in the retention efficiency study. The challenge solution was passed through each individual syringe filter and a 3mL eluted aliquot was collected and analysed at 272nm. Three different filters from each of the three lots were tested (that is nine filters from each manufacturer were individually analysed for effluent latex sphere content).

The HPLC was utilised for the column-plugging study. Column life was evaluated by comparing initial backpressure to backpressure after injections. A new LUNA C18(2) 00A-4252-Y0 column (S/N: 160111-3) was installed. The outlet of the column was disconnected from the detector and allowed to run to drain. This modification allowed quicker injections for a more efficient determination of column backpressure. Acetonitrile:Water (35:65, percentage by volume) was used as a mobile phase, with a flow rate of 1mL/min. Column temperature was controlled at 25°C by a column heater. The system was set to automatically inject 50μL each time. The column-plugging solution consisted of

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0.05% (by weight) 0.45µm average diameter latex spheres in 0.002% Triton X-100 solution, which was found to plug the column with a reasonable number of injections. The first step was to inject this solution without any filtration to see how long the column would last. After the column was plugged (that is, column backpressure reached 3500 psig), a new Luna C18(2) 00A-4252-Y0 column (S/N: 159485-4) was installed. This time the same latex-sphere solution was subjected to filtration using the first set of filters. Thirty samples were generated with 30 filters from the first supplier (10 from each of the three lots). The injections were carried out from sample vial one through vial 30 and then repeated in this sequence. The column backpressure was recorded with the number of injections. This procedure was repeated with new columns, S/N 159742-3 and 159485-3, for studies using filtrate from the products supplied by the remaining two vendors, respectively.

**TEST RESULTS AND DISCUSSION**

Latex Sphere Retention For Filters From Three Manufacturers

Tables 1-3 list latex sphere retention capabilities of filters from the three vendors. The filters with the best performance are able to retain an average of 94.9% of the 0.45µm average diameter latex spheres. By comparison, the filters from the remaining two suppliers can only remove averages of 90.5% and 33.0% of the 0.45µm average diameter latex spheres respectively. Based on the three lots tested, the first filters evaluated also show greater lot-to-lot consistency with relative standard deviation (RSD) of 3.5%.

These syringe filters from different manufacturers may appear similar and are all assigned a pore size rating of 0.45µm from the manufacturer, but the test results show that they perform differently.

Function of Filtration to Prolong HPLC Column Life

Figure 1 depicts the relationship between column backpressure and number of injections. Without filtration, the column failure due to plugging occurred after only 21 injections. After the 0.05% latex-sphere solution was passed through the filters from vendor two and vendor three, the columns were plugged after 37 and 487 injections, respectively. In addition, the effluents from filters supplied by both vendors were hazy, indicating the presence of significant numbers of latex
spheres. When the 0.05% latex-sphere solution was passed through the filters from vendor one and injected to the HPLC system, the column backpressure did not increase after even 972 injections. The clear effluents from the filters suggested a more successful retention of latex spheres.

Column Plugging Mechanism
When particulates in HPLC samples are not effectively filtered out before injection, they may build up on/in the column inlet frit and/or accumulate in column packing, which will lead to increased back pressure. In this study, the frit pore size is 2.0 μm. Therefore, some unfiltered 0.45 μm latex beads may be lodged in the tortuous path within the frit or retained by the frit due to ‘bridging’ effect; others may pass through the frit and reach the column packing.

Figure 2 illustrates the flow path between the 5 μm packing materials within an HPLC column. Based on mathematical calculation, for 5 μm packing materials, latex beads with diameter greater than 0.72 μm could not pass through the packing materials. Some of the unfiltered 0.45 μm latex beads, after going through the frit, may be trapped between the packing materials and block the flow path, thus increasing column back pressure; some of them may find a way and pass through the packing materials.

CONCLUSION

Among 0.45 μm rated filters from three manufacturers, there is a clear difference in filter efficiency, column protection ability and lot-to-lot consistency (based on the three lots tested). This study shows that it is imperative that samples are filtered prior to introduction into an HPLC system. Apparently equivalent filters from various manufacturers with the same removal rating differ in capabilities. Using the most efficient of these filters extended the column life by 46 times (compared to no filtration), with no increase in column backpressure.

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