Guarding Column Lifetime

It is commonly accepted that using a guard column prolongs the lifetime of an analytical column. Placing a barrier in front of an expensive analytical column will inevitably slow down the attack on that column. Unfortunately most HPLC practitioners do not act in their company's best interest by purchasing guard columns. The reason for this may be that working with a guard column adds a degree of complexity. The user now has to look after two columns. Is each of them working properly? Is the packing material of the same lot as that in the analytical column? Does it actually matter? Does it make financial sense to protect your analytical column with a guard column? The following discussion and data illustrates the beneficial effect a guard column can have on the lifetime of an analytical column.

This paper will focus on TSKgel G3000SW$_{XL}$ and SuperSW3000 size exclusion columns, which are sold by Tosoh Bioscience and are used widely by chemists working in the biopharmaceutical industry. To determine the value of a guard column, consider these points:

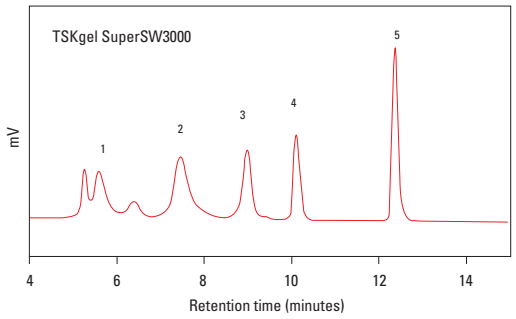
1. After installing a TSKgel SW$_{XL}$ guard column in front of a TSKgel G3000SW$_{XL}$ column, the analysis time increases by about one minute assuming that the column set was run at 1mL/min and that this was done as well when the analytical column was operated by itself. This answer is derived by first calculating the volume of the 6mm ID x 40mm guard column (1.13mL). As for analytical columns, about 80% of the empty guard column volume is taken in by mobile phase. By multiplying 1.13 by 0.8, this shows that roughly 1mL of additional volume of the sample has to travel at a flow rate of 1mL/min before reaching the detector. Instead of an analysis time of 13 minutes, cycle time is now 14 minutes, or 8% longer, which translates into completing 34 instead of 36 samples during an 8 hour shift.

2. There is an additional cost associated with guard columns. Before using an analytical column, a small molecular weight solute such as p-aminobenzoic acid (pABA) ought to be injected to check the physical integrity of the packed bed by measuring the efficiency and peakshape of pABA and compare these numbers with the data that is listed on the Inspection Data Sheet (IDS) that is supplied with each column. Assuming the column meets suitability criteria, the column is ready for sample analysis. But now we first need to connect the guard column to the analytical column. As when installing an analytical column, it is best to first briefly flush the guard column in normal flow direction and have the effluent go directly to waste while bypassing the detector before connecting the analytical column to the guard column and detector. To be certain that the combined column set passes suitability criteria, again run the pABA test. *This run constitutes additional time that you cannot use to analyze a real sample.*

3. Sometime after the start of using the column set, the efficiency of the separation may decrease. This is an indication that the guard column may be losing its effectiveness. The guard column should be removed and the analytical column tested again (with a specific sample this time) to make sure that it is still functioning properly. This also constitutes extra time in which a sample cannot be analyzed. For the sake of this argument, it is assumed that the analytical column is still going strong (due to the protection by the guard column). Try cleaning the guard column on another HPLC system (actually a simple pump would suffice) by using the recommended cleaning procedure for that guard column, while installing a new guard column in front of the analytical column. Of course, the new guard plus existing analytical column needs to be checked again to ensure that the column set is functioning properly. This however does not constitute additional time, since a new analytical column would have to be installed if a guard column had not been used and that new column would have had to be checked as well.

4. Turning now to *the benefit side* of the equation, it is easy to come to the conclusion that the increase in analysis time and the additional time invested in checking the performance of the guard and analytical columns will be more than offset by the prolonged lifetime of the analytical column.

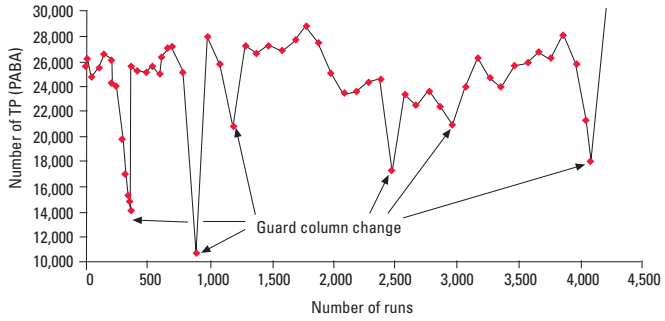
Figure 1.



Column:	TSKgel SuperSW3000, 4µm, 4.6mm ID × 30cm
Mobile Phase:	0.2mol/L phosphate buffer, pH 6.7
Flow rate:	0.35mL/min
Detection:	UV@280nm, micro flow cell
Samples:	standard proteins (5µL)
	1. thyroglobulin (0.5g/L)
	2. γ-globulin (1g/L)
	3. ovalbumin (1g/L)
	4. ribonuclease A (1g/L)
	5. *p*-aminobenzoic acid (0.01g/L)

To illustrate this fact, a study was conducted that demonstrates the beneficial effect a guard column can have on the lifetime of an analytical size exclusion column. The column used was a TSKgel SuperSW3000, a 4.6mm ID x 30cm column packed with spherical 4 micron particles with an average pore size of 250 Angstrom. In the study the column efficiency was monitored for more than 4000 injections of a mixture of protein standards. *Figure 1* shows a representative gel filtration chromatogram for standard globular proteins on a TSKgel SuperSW3000 column.

Figure 2.



Column:	TSKgel SuperSW3000, 4µm, 7.8mm ID x 30cm + guard column
Mobile phase:	0.1mol/L phosphate buffer + 0.1mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35mL/min
Detection:	UV@280nm, cell volume 1µL, response time 0.2sec.
Temperature:	ambient
Samples:	γ-globulin (156kDa), 1mg/mL mobile phase ovalbumin (43kDa), 1mg/mL mobile phase cytochrome C (12.4kDa), 0.5mg/mL mobile phase PABA (137kDa), 0.01mg/mL mobile phase

The graph in *Figure 2* shows how column efficiency of a TSKgel SuperSW3000 column varied as a function of the number of injections. The experiment was run by injecting a standard mixture of proteins every 20 minutes and measuring column efficiency for a small molecular weight compound (p-aminobenzoic acid) after every 5 injections during the first 500 injections. After 500 injections, column efficiency was checked roughly every 100 injections. Throughout the study, the analytical column was protected by a 4.6mm ID x 3.5cm TSKgel SuperSW guard column, which is packed with the same support as that contained in the analytical column.

Note that although column efficiency decreased on multiple occasions throughout the study, efficiency (and peak symmetry as well; data not shown) was restored by simply replacing the guard column! In a separate study performed in 2007, the stability of two TSKgel G3000SW_{XL} columns for more than 1000 injections each of a monoclonal antibody solution was tested. In this study it was also found that column efficiency could be restored each time after replacing the guard column.

Remember at all times that a guard column is nothing but a small analytical column. Anything that fouls the analytical column will also foul the guard column. Samples may contain highly retentive contaminants that may never elute from the column and restrict flow through the pores or irreversibly adsorb on the stationary phase. Also, seals in the pump and in injection valves can throw small particles that can clog frits, which may cause efficiency to decrease while not always giving rise to high pressure. If columns are failing after a limited number of injections, it is better to first find out what is causing the short lifetime of the analytical column. The reason may be sample-related or could be due to the mobile phase conditions, but it could also be related to sub-optimized HPLC system components.

As demonstrated in this note, when an analytical column is properly protected, the lifetime of the column will be enhanced and savings in time and money will be realized.

TOSOH

TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com