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This morning, I was watching a video clip forwarded by one of my motorcycle enthusiast friends. This was a video clip of a MotoGP race in which one of the riders loses control over the bike and accidentally slipped. The riders behind him, not anticipating this sudden event, crashed into each other leading to a pile of bikes. Luckily the crash was not fatal, and all the riders survived. When I tried to understand the reason as to how these riders got saved, I noticed that it is because of their protective gear that they were wearing.

The safety of the rider is of utmost importance when it comes to motorcycle racing, so they protect themselves by using the best protective gear, by anticipating the worst. Similarly, a HPLC column is the main component in LC chromatography because it is the column that is responsible for the separation of the analytes in a matrix. Protecting and taking care of the column ensures that we not only get reliable results but also trouble-free operations. We all know that a HPLC column has a finite life, but how do I ensure that I get maximum life?

To answer this question, let us first look at the possible ways in which a column can get blocked?

Sample Matrix:

Samples introduced onto the HPLC column are often diverse and complex. These samples have many components that might not be compatible with the HPLC method conditions. Injecting such samples without proper sample preparation/pretreatment might lead to the

clogging of the column.

Residual proteins and highly hydrophobic compounds like lipids may also not be completely eluted out during reversed phase separations, these tend to slowly get adsorbed onto the column and cause issues.

Incompatible sample diluent and mobile phase:

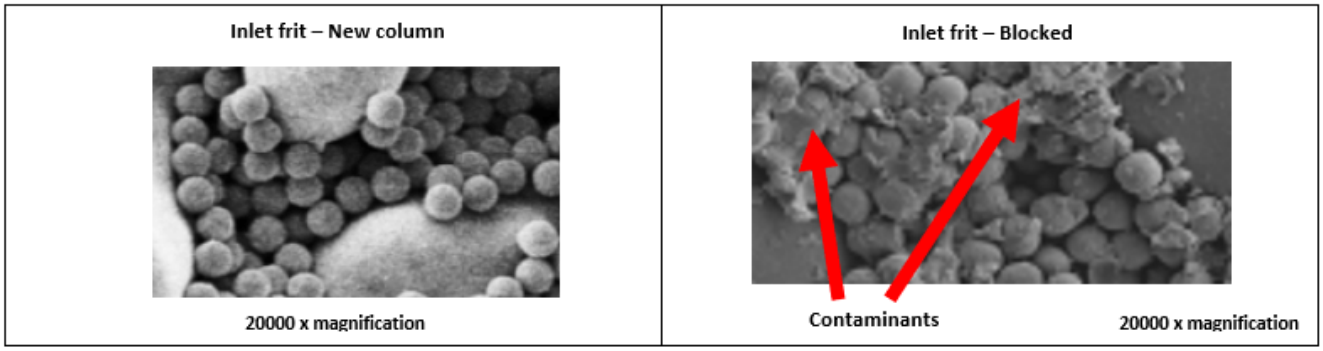
If the sample diluent is not compatible with the mobile phase then when a sample is introduced into the mobile phase, there is a possibility of the sample precipitating. This is often seen if the diluents are too polar or too non-polar compared to the mobile phase.

Mobile phase buffers:

Buffers are commonly used as mobile phase in HPLC. It is important that these buffer salts be filtered prior to use. Sometimes, due to the difference in the temperature of the laboratory, some of the buffer components tend to precipitate. Using too high concentration of buffer also leads to precipitation.

Fragments from worn out pump seals/rotor seals of injector:

Pump seals and injector rotor seals are often made of rubber material. Due to normal wear and tear, these components tend to generate some debris into the solvent line. If periodic maintenance of the pumps and the injector ports are not done, the debris tends to reach the column frit and block the column.



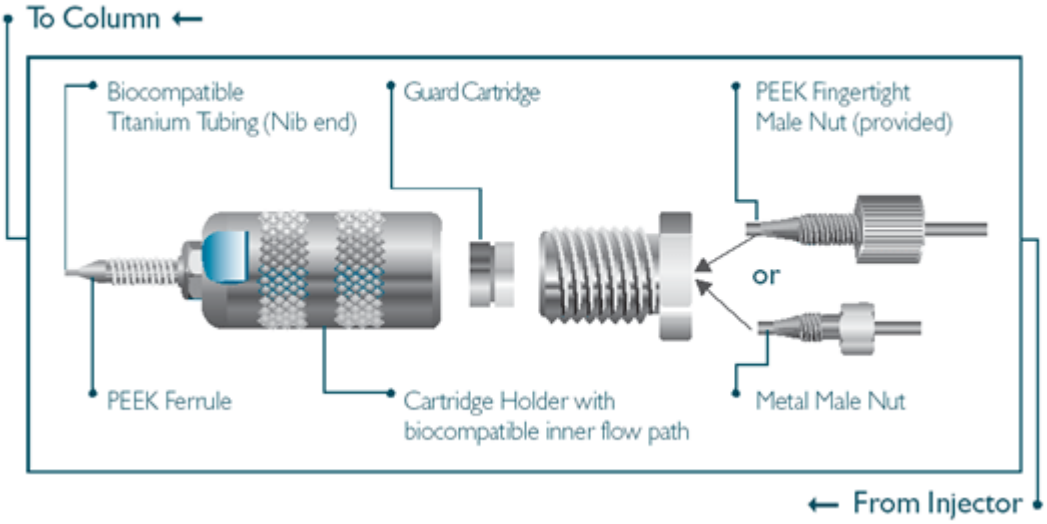
Now that we know the possible ways in which a column can get blocked. How do we protect it?

To protect HPLC columns (analytical and preparative) from contamination, like particulates from the injection, unfiltered mobile phase components or fragments from worn pump seals/ rotor seals from the injector, it is recommended to use a guard column.

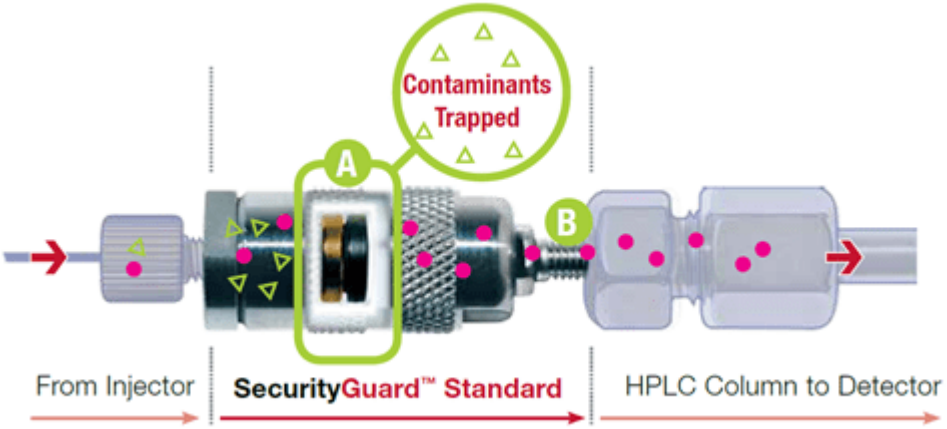
The next obvious question is, what is a guard column?

A guard column is essentially a mini column, ideally packed with the identical packing material as that of the analytical column and is connected before the analytical column.

These columns come in various dimensions to match the requirement of the columns. The frit in the guard columns is like the one in the analytical column. This would mean that anything that might potentially block the analytical column would be blocked on the guard column. This block can then be removed by simply replacing the guard cartridges, thereby protecting the life of the analytical column.

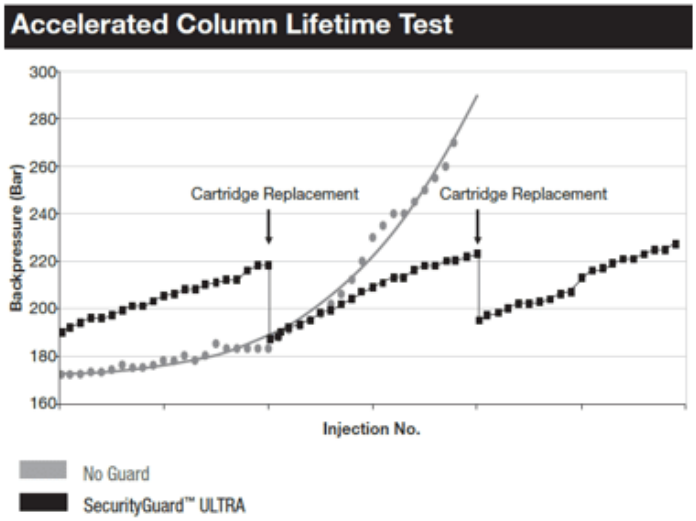


Components of the Security Guard™



Cut away view of the Security Guard showing the Guard cartridge that can be replaced easily when contaminated.

To understand the role of a guard column on the column life we have performed an accelerated lifetime test using an endogenous biological matrix injected onto a core-shell column (Kinetex 2.6 µm C18 50 × 4.6 mm column Phenomenex, Inc. Torrance CA, USA). With the unprotected column (grey dots, picture below), sequential injections of the matrix lead to a steady and irreversible increase in backpressure. Without SecurityGuard ULTRA column protection, the increase in backpressure became exponential. This increase in backpressure would eventually lead to degraded chromatography, including band broadening and possibly peak splitting. As a result, method sensitivity, quantitation, and peak identification may also be adversely affected.



However, column lifetime was greatly extended by using the SecurityGuard ULTRA (black boxes, pictured above). In this case, sequential injections of the matrix still lead to an increase in pressure, but this was due to the particulates being captured in the SecurityGuard ULTRA itself, rather than in the UHPLC/HPLC column. Thus, by simply replacing the SecurityGuard ULTRA cartridge at regular intervals, backpressure returned to starting levels and effective column lifetime was greatly extended.

When do I replace my cartridges?

Visually inspect the surface of the cartridge's packing material any time, without disturbing the packing bed. Now you can easily monitor visual contaminant build-up and change your guard cartridge before it's too late! If your contaminants are colorless, replace the cartridge as often as needed to maintain chromatographic performance (e.g., when resolution decreases by 10% and/or when efficiency/peak widths decrease by 20%) or if the backpressure of the system increases by more than 20%.

What are the features that I should look for while purchasing a guard column?

The packing material (stationary phase) of the guard cartridge should be the same as that of the analytical column so that there is no change in the selectivity.

The ID of the guard cartridge should be the same or smaller than the ID of the analytical column to minimize increased band broadening. Always use a guard with ID closest to that of the column.

The length of the guard column should be as minimal as possible to avoid additional dead volumes.

The cartridges should be easy to replace whenever necessary.

Phenomenex offers three types of guards to cater to most of the columns that are being used.

SecurityGuard Standard: Recommended for use with all non core-shell and ≥ 3 µm particle columns ((<5,000 psi /345 bar)*), they are compatible with virtually all HPLC columns.

SecurityGuard ULTRA: Recommended for use with all core-shell and/or < 3 µm particle columns (< 20,000 psi / 1,378 bar)

SecurityGuard PREP: Recommended for use with Prep HPLC columns with 9.0 to 49.0 mm internal diameter (ID)

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