

Choosing the Most Appropriate Column Protection

Key Words

- Column Protection
- Guard Columns
- Javelin Guards
- UNIGUARD System

Introduction

Column protection has become more important with the ever increasing demand to analyze larger, more complex sample mixtures, often with very limited time scales. Problems with the column or the need to replace it always seem to occur at the least opportune time. It is much easier and less expensive to simply replace a guard cartridge or a filter, than to replace a column. Thermo Scientific Corporation has a range of guard designs that make column protection a simple process.

In this short bulletin, we will review the different column protection devices available. In doing so, we also highlight some of the typical questions and concerns that our Technical Support Group is regularly asked. Figure 1 summarizes this selection of guards and filters and some of their important features.

Typically column “Protection Devices”:

- Prolong column life
- Remove particulates that will clog the analytical column
- Remove compounds, including ions, that will be strongly adsorbed by the packing

UNIGUARD holder and cartridge

- 1 cm protection
- Direct connection to column
- Replaceable cartridge



Javelin Guard

- Direct-connect to column
- Finger-tight connection
- Disposable stand-alone guard



Stand-Alone Guard Cartridge Holder

- 1 cm protection
- In-line connection
- Convenient drop-in replacement cartridges



Figure 1: A selection of guard and filter designs

What Roles Do Guard Columns Play in Column Protection?

Guard columns are singly the most important column protection device that you can install. Column damage primarily occurs near the column inlet, where sample matrix adsorption, dissolution of silica or voiding will occur. The guard column replicates the top small portion of the column (usually 1 cm in length), so that any damage that occurs is isolated at the guard, sparing the inlet area of the analytical column. Regular replacement of the guard will increase the lifetime of the column significantly. Different types of guard systems and protection systems are available, and these vary from drop-in type guards such as the UNIGUARD system to the Javelin guard system (Figure 1). These are discussed in more detail below.

When Should I Use a Guard Column?

- Do you run contaminated samples?
- Is there a high sample throughput required from your lab?
- Do you run samples at elevated temperatures?
- Do you get high back pressure after modest column usage?
- Do you find that after a few injections some of your peaks appear to be retained slightly differently?

If the answer to any of these questions is yes, using a guard system will save you considerable effort and money. It simply makes sense to protect your analytical column whenever you can.

The lifetime of an HPLC column can vary significantly depending on its handling and specific application. The main factors affecting the long-term performance are the mobile phase composition, temperature, the number of injections made, the operating pressure and the nature of the sample matrix.

The most common problems are sample related. Contaminants in a sample matrix can build up on the inlet frit of the analytical column leading to an increased column operating pressure often resulting in loss of performance. Alternatively, some impurities in the analyte mix can become irreversibly adsorbed to the stationary phase causing its surface properties to change. This can lead to a change in the chromatographic performance and selectivity of the column. The use of guards largely overcomes these difficulties.

What Packing Should I Use in the Guard? Is There a Universal Guard System?

The choice of packing is very important to prevent loss in performance. Where possible, the packing used in the guard should match that of the primary HPLC column. Figure 2 demonstrates the problem that can occur when the guard and analytical columns are incorrectly matched.

Thermo's UNIGUARD holder and drop-in guard cartridges are also good choices. The UNIGUARD design is suitable for many packings not listed in our catalog. In this case we will be happy to help you match the appropriate guard cartridge to complement your column

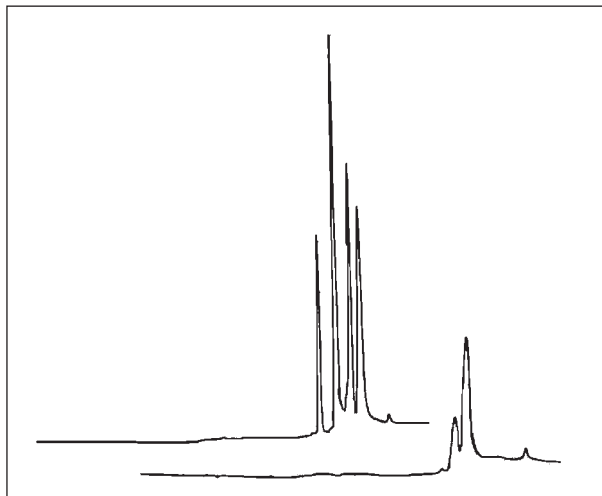


Figure 2: Effect of wrong guard column on protein separation. Upper chromatogram is column alone. Lower chromatogram is column with guard.

How Will the Addition of a Guard at the Front of My Column Affect the Retention Time and Performance of My HPLC Column?

Typically, the use of a 1 cm guard will add approximately 6% retention time to a 150 x 4.6 mm column and approximately 4% to a 250 x 4.6 mm column. The effect of a 2 cm guard column is shown in Figure 3.

No loss in performance is observed when attaching the guard column to the primary column, provided the same packing or a suitable alternative packing is employed.

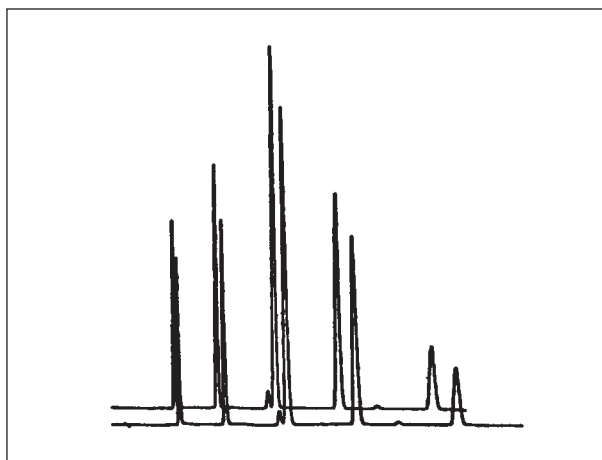


Figure 3: Effect of a 2 cm Guard Column on Column Retention. Upper chromatogram is without guard. Lower chromatogram is column with guard.

How Much Longer Will My Column Last if I Use a Guard System?

This is largely dependent on the type of samples and sample matrix and the mobile phase conditions (i.e., buffer type and pH) used. To give some indication of lifetime, we have monitored the lifetime of the UNIGUARD system (Figure 4).

A 1 cm guard should not significantly increase the pressure of the system when first installed. If the guard is trapping particles or impurities throughout each run, an increase in pressure is to be expected. The amount of increase is determined by observing the pressure increase over a number of sequential sample injections (see Figure 4). Figure 4 shows the total system pressure might change after a number of injections, i.e. upon installation and over a period of gentle use.

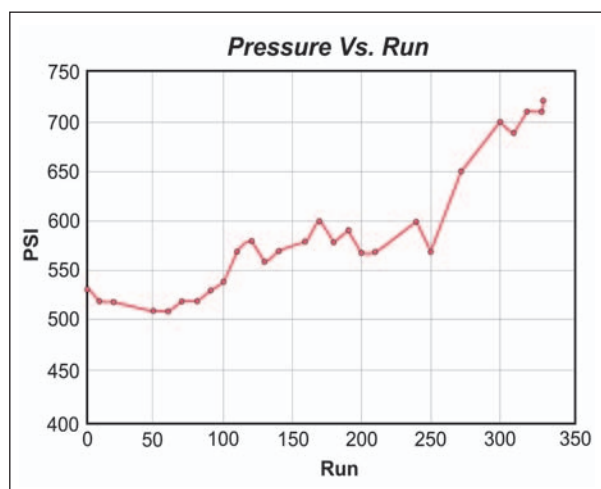


Figure 4: UNIGUARD drop in-guard cartridge pressure studies run using low molecular weight chemical standards over the number of injections indicated.

My Samples are Very Contaminated – What Size Guard Column Should I Use?

When choosing a guard, take into account the level of impurities in your samples. For example, some samples that appear clear and clean to the eye may contain plasma proteins from a biological sample, crop extract or urine impurities. In most cases a 1 cm guard will provide adequate protection. If your samples are very contaminated and you find that you need to change your 1 cm guard often, then a 2 cm guard will offer more capacity and can reduce the frequency with which the guard needs to be changed.

What are the Signs that My Guard has Reached the End of its Useful Life?

Typically there are several symptoms that will alert you that it is time to change your guard:

- Split peaks
- Increased back pressure
- Change in chromatographic selectivity
- Loss in resolution

Figure 5 illustrates how simply replacing the guard column can bring back the original performance of the column. Note: This is exactly the kind of damage that would have occurred to the primary analytical column had the guard column not been in place. Guard columns can offer real savings in cost and time.

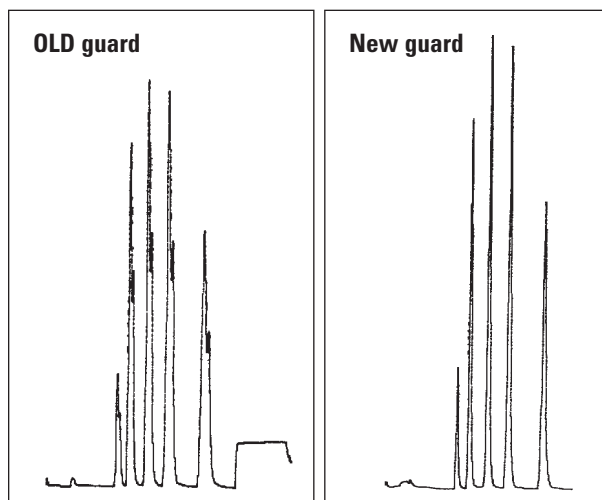


Figure 5: Split peaks from contaminated guard

Are There Different Types of Guard Hardware Design Available?

We offer a range of guard designs. These include:

- Direct-connect – finger-tight
- Traditional stand-alone design

The direct-connect designs, UNIGUARDs and Javelins, can be installed at any time by the chromatographer and are very convenient. They can also be used on many different manufacturers' columns.

In cases where a guard is installed before the injector or is used in a manner where tube connection is more convenient, in-line designs are available. See Table 1 for a summary of guard designs that are available.

Product	Packing Bed Length	Connection	When to Choose	Types Available
UNIGUARD System	1 cm	Direct Connect	First choice for most reversed phase and normal phase applications. Uses convenient replaceable cartridges	Choice of UNIGUARD drop-in cartridges in many brands of packings. Most economical design.
Javelin Guard	2 cm 1 cm	Direct Connect to column	Choose when 2 cm of protection is needed for more capacity and direct connection is desired. Entire guard is replaceable	A variety of Thermo brands
Stand-Alone Guard Cartridge Holder	1 cm	In-line	First choice when in-line design is desired. Uses the same convenient drop-in guard cartridges used in the UNIGUARD system	Many Thermo brands of packings

Table 1: Summary of Guard Designs

In addition to these offices, Thermo Electron Corporation maintains a network of representative organizations throughout the world.

- Australia**
+61 2 8844 9500
- Austria**
+43 1 333 50340
- Belgium**
+32 2 482 30 30
- Canada**
+1 800 532 4752
- China**
+86 10 5850 3588
- France**
+33 1 60 92 48 00
- Germany**
+49 6103 408 1014
- India**
+91 22 6742 9434
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