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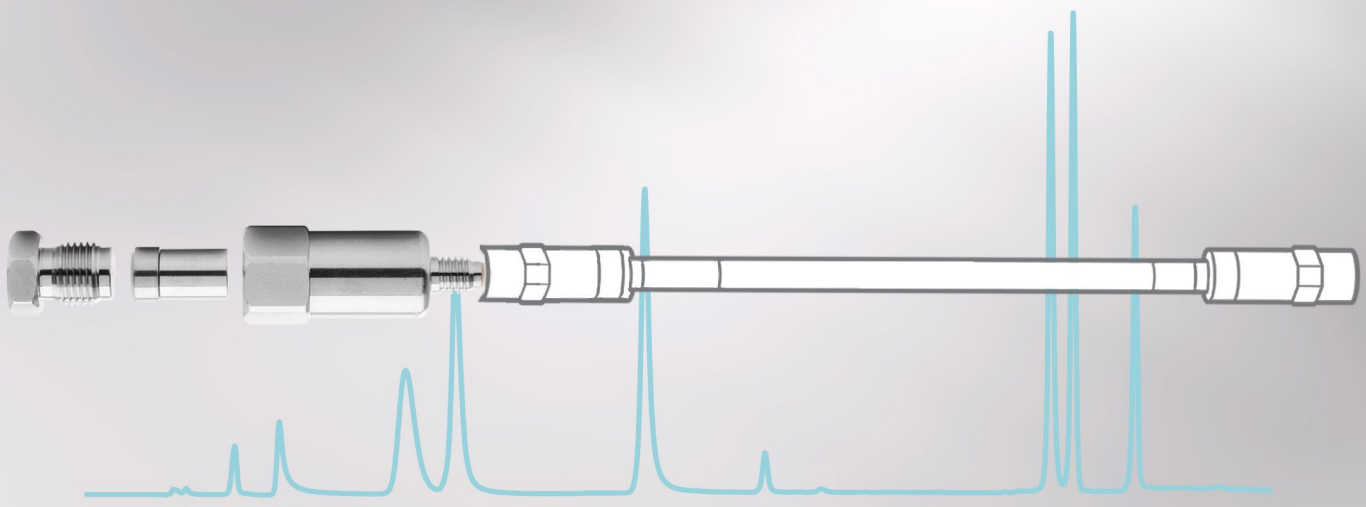

Chromatography

The effects of using a guard column on chromatography

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Keywords

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Goal

With this application, we show the benefit of using a guard column on analytical methods as well as proving its minor effect on the chromatography.

Introduction

Guard columns are short columns or cartridges installed prior to an analytical or preparative HPLC column, which act as a filter to prevent particulates from entering the main column and trap any chemical contaminants with a high affinity for the stationary phase. This ensures the integrity of HPLC results and increases data reproducibility by removing chemical impurities and other physical contaminants that can act as blockage. Using a guard column or holder/cartridge system can, thus, greatly increase the lifetime of your main column.

Historically, a guard column was a shorter column placed in front of the analytical column. The size was about 50 mm long as the guard meant to have as little impact on the chromatography as possible. With the improvements happening on the instrumentation side and the aim to create shorter and more efficient methods, the guard column became a factor for increased dead volume and essentially affected the overall chromatography, widening peaks and shifting retention times to a larger degree.

The newer technology guard holders and cartridges, such as the Thermo Scientific™ Uniguard™ direct-connection guard cartridge holder, now help achieve protection and longer lifetime of the more modern HPLC columns at reduced cost and downtime and with lower dead volume.

Despite the widely accepted knowledge that a guard column in front of the HPLC column will help protect the column and increase the lifetime, the impact of the guard column is often underestimated, or the column is simply not used due to the fear of its impact on the overall chromatography. Here, we clearly describe the positive impact of using a guard column and demonstrate the small impact it has on the analytical results.

Experimental

Sample

For this experiment, we selected a paracetamol application, where the sample was common acetaminophen tablets (paracetamol) from a local pharmacy. Further materials are listed below.

Column and guard

- Thermo Scientific™ Hypersil GOLD™ C18 selectivity HPLC columns (3 µm × 3 mm × 100 mm), P/N 25003-103030
- Uniguard direct-connection guard cartridge holders, P/N 852-00 (2.1 mm to 3 mm i.d. guard columns)
- Guard cartridge, P/N 25003-013001

Instrument

- Thermo Scientific™ Vanquish™ Horizon UHPLC system
  - Diode Array Detector HL, P/N VH-D10-A
  - Split Sampler HT, P/N VH-A10-A
  - Binary Pump H, P/N VI-P10-A

Standards, reagents, and consumables

- Milli-Q™ water, MilliporeSigma™ Milli-Q Gradient A10 water purification system
- Methanol, Optima™, Fisher Chemical™, P/N A454-4
- Acetic acid glacial, ≥99%, Fisher Chemical™, P/N A/0360/PB17
- Paracetamol standard, EDQM, P/N P0300000
- Thermo Scientific™ SureSTART™ Specification Certified Screw Vial and Cap Kits, Level 2 High-throughput Applications, P/N 6AK92W

Sample preparation

- **Standard preparation:** Dilute 10 mg of acetaminophen RS with diluent to 100 mL. Dilute 100 µL standard with 900 µL diluent.
- **Sample stock solution preparation**: Smash 10 tablets of 5 mg paracetamol. Mass dilute with diluent to 50 mL. Sonicate for 5–10 minutes. Centrifuge at 4,000 xg for 5 minutes.
- **Sample preparation:** Dilute 1 mL of sample stock supernatant to 10 mL with diluent.

Method conditions

Parameter	Value
Mobile phase A	1% acetic acid in water: Dilute 20 mL of acetic acid to 2 L with Milli-Q water.
Mobile phase B	Methanol
Diluent	10:90 Methanol:water
Column temperature	40 °C
Flow rate	0.5 mL/min
Injection volume	10 µL
Gradient	Time (min) %A %B 0 90 10 3.44 90 10 3.53 20 80 5.16 20 80 *Equilibrate for 4 minutes

Results and discussion

We ran the paracetamol method on approximately 100 samples per day for 55 days without a guard holder/cartridge in front of the analytical column. As we analyzed the results (Figure 1), we saw great deterioration in the peak asymmetry after day 29. By day 33, the asymmetry was outside pharmacopoeia method limitations. Figure 2 shows how pressure behaved throughout the experiment. We saw an increase in pressure without a guard across the 55 days. To assure peak asymmetry was maintained within the limit, we set the guard cartridge exchange date to day 20, prior to the expected deterioration, and reran the experiment with the guard holder and cartridge, this time stopping the experiment at day 40. Throughout this experiment, the asymmetry did not rise or go outside of the limit and the pressure remained relatively level.

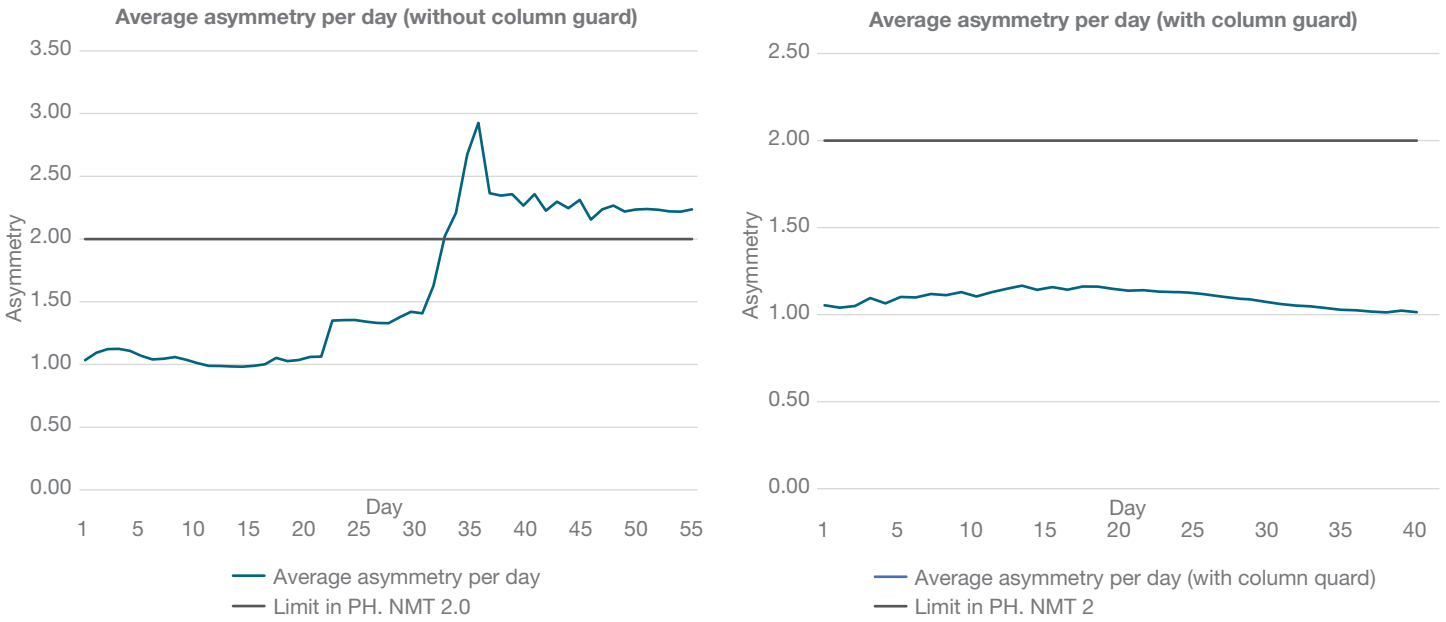

Figure 1. Average asymmetry per day for method run without (left) and with (right) a guard holder with cartridge

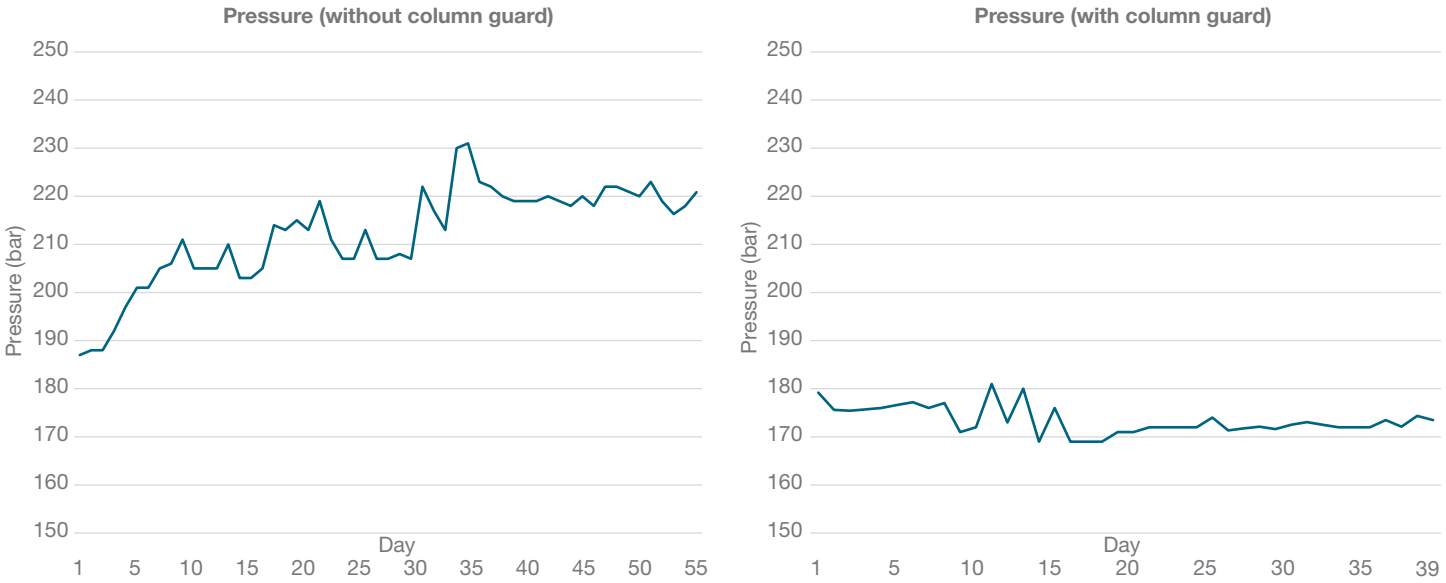

Figure 2. Average pressure per day for method run without (left) and with (right) a guard holder with cartridge

Electron microscope images were taken of the guard cartridge before and after use to evaluate the probable damage to the analytical column if a guard cartridge is not used. The images in Figure 3 show a clear impact on the cartridge. A dark indentation formed on the surface from contaminants in the introduced samples, and when zooming in on the dark areas, crushed materials can be seen. The crushing of the material happens due to the pressure that forms at the head of the column. This crushed material at the head of the column increases dead volume in the column, which in turn degrades the chromatography and affects the peak shape. Being able to exchange this material assures uniform peaks shape across time.

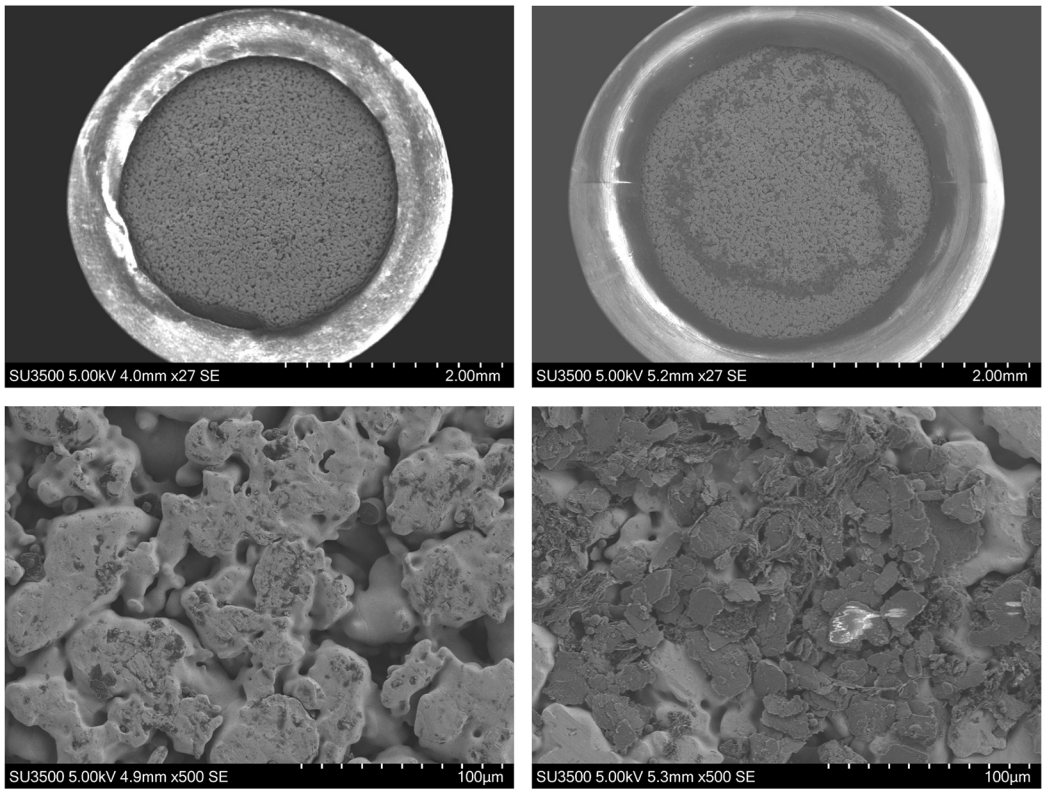

Figure 3. Electron microscope images

Figure 4 shows two chromatograms—one of an injection where a guard column is used and one where it is not used. There is minimal impact of the guard column on the overall chromatography, with only a small shift in retention time.

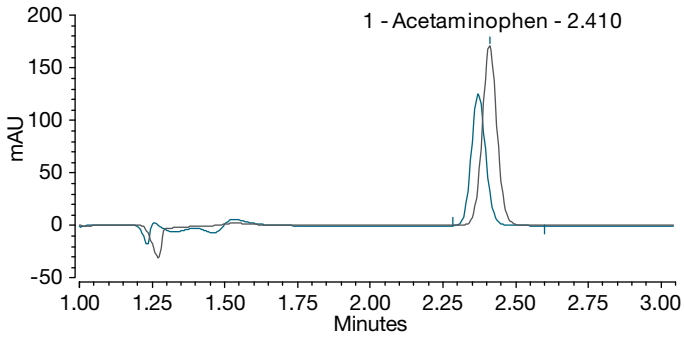

Figure 4. Chromatograms of acetaminophen on an analytical column (blue) and on an analytical column coupled with a guard column (gray)

Conclusion

Our experiments show clear advantages to utilizing a guard column prior to the analytical column, in both visible benefits to the analytical column and in data, such as peak asymmetry. We have also shown that the impact on the overall chromatography is relatively small, and thus, a guard column can be used without concern for validation.

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