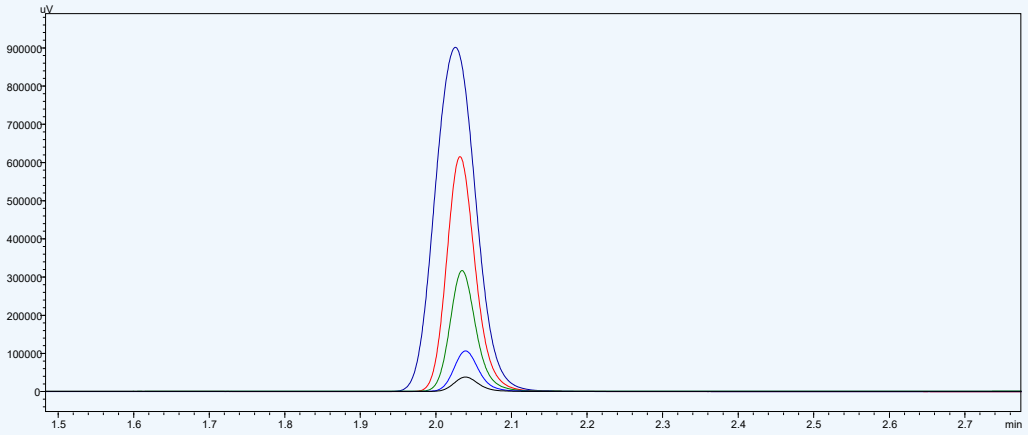
Investigating Back Pressure in EP 10.7: Paracetamol Sample Loading Effects

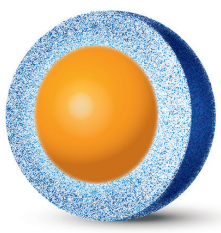
Following the revised EP 10.7 method as written has proved challenging due to the noted increase in back pressure which arises after fewer than what would be normally expected injections on column. In our study of these reports, it was found this can be attributed to sample overload and sample solubility issues.

A column gets overloaded because there are more solute molecules seeking active sites than there are available sites in the column. In other words, there are a finite number of active sites on the stationary phase of a column available for the solute molecules to transiently bind to.[1] Overloading the column with sample can cause several issues with your chromatographic results including increased peak widths, decreased resolution between peak pairs, incorrect retention times, and damage to the column itself.

A change in sample load or injection volume should only affect the peak height and area of the chromatogram. Column overload can be attributed to the surface area of the particles; however, the compound of interest and the method conditions being used also play an important role. For example, Figure 1 shows how paracetamol peak shape is affected as the sample injection volume is increased. Peak widths will increase and the retention times will shift as too much sample is loaded on column.

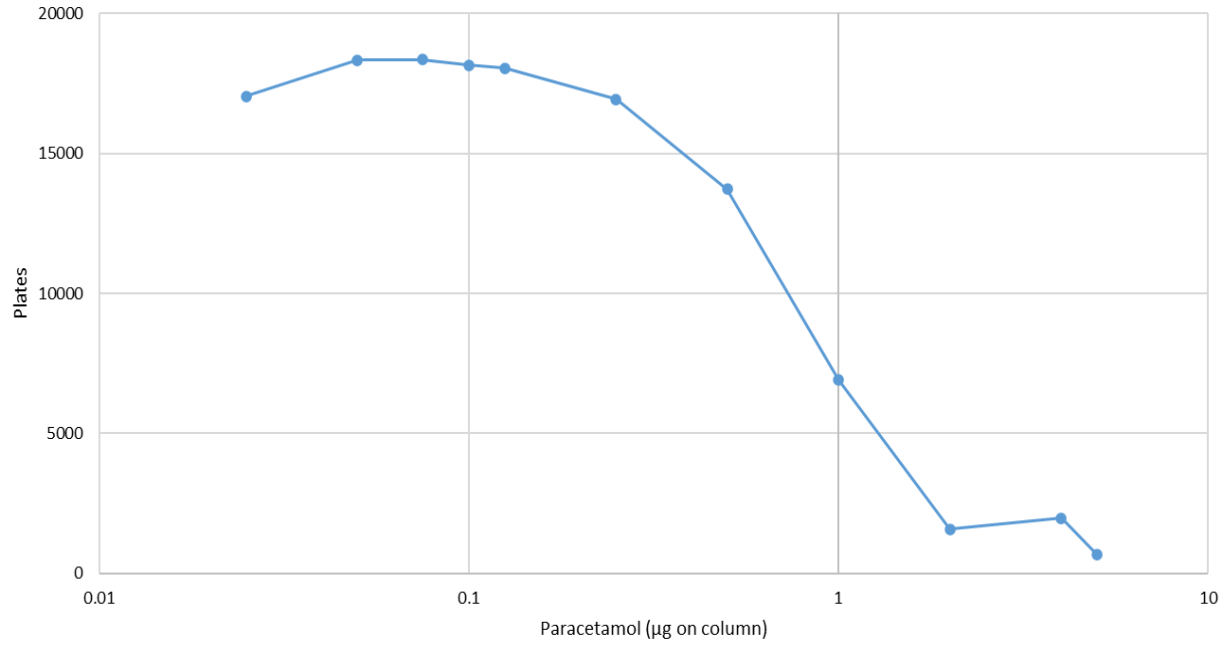
Figure 1: Effect of injection volume on paracetamol peak shape





Due to the wider peak widths and sample loading effects the columns plates/ efficiency will decrease as too much sample is loaded on the column. Figure 2 shows how the plates decrease as sample load increases for paracetamol following conditions listed in EP 10.7 (Paracetamol and Related Substances).

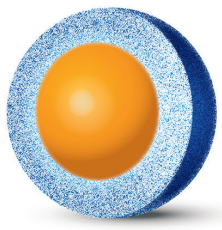
Figure 2: Effect of sample load on paracetamol plates using a HALO 90 Å C18, 5µm 4.6 x 150 mm following EP: 10.7 conditions.



Some things to look out for when a column is being overloaded are wide peak widths and even flat top peaks which usually means that the detector is being oversaturated. To test if the sample load is too large, dilute your sample in half and re-run the sample. If there is no significant change in retention time, efficiency, or resolution, then the sample load was not too large.

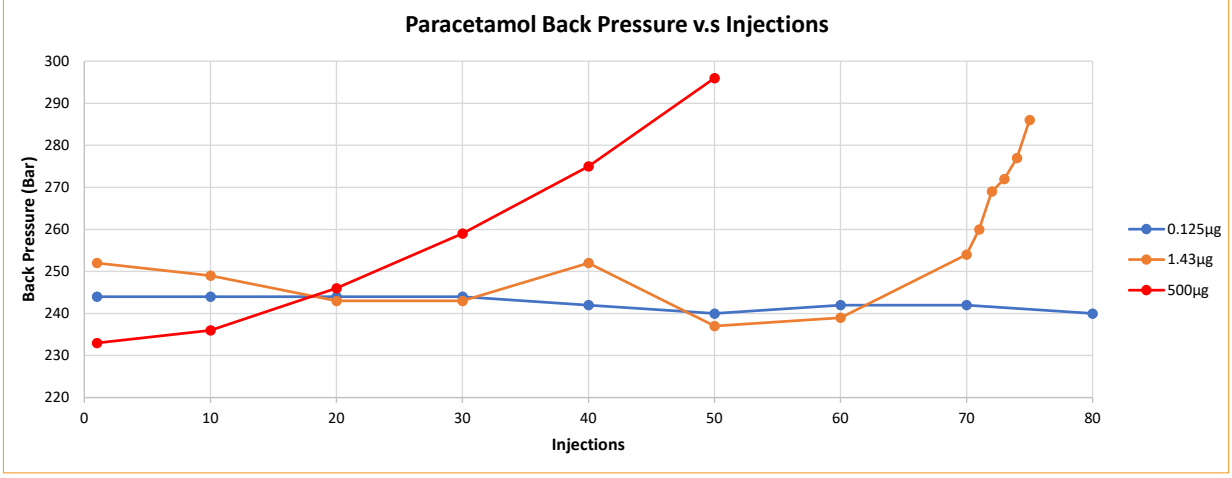
For typical separations on columns with lengths of 50 to 250 mm, and an internal diameter of 4 to 5 mm, the mass of individual compounds in the sample should be limited to ≤ 50 µg, with a sample volume ≤ 25 µL (when the mobile phase is used as the injection solvent). For smaller diameter columns, sample size should be reduced in proportion to the square of column diameter.[2]

Another concern is if the sample load is too large it will damage the column, including plugging up the frits. It is important to match the sample solvent with the initial starting conditions of your method in order to avoid the sample crashing out. This can lead to poor peak shapes and an increase in back pressure over time. The example in Figure 3 shows results from method EP 10.7 (Paracetamol and Related Substances) run



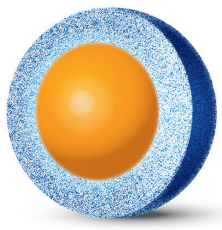
at different sample loads on a HALO 90 Å C18, 5 µm 4.6 x 150 mm column which is the required particle size and column dimension for the method. As demonstrated, back pressure is observed over a series of injections and the column with the highest sample load (500 µg on column per EP method 10.7) shows a significant increase in back pressure while the recommended sample load from Figure 2 (0.125 µg) shows stable back pressure throughout the series of injections. The increased back pressure results from plugging of the inlet frits which is due to sample overloading and the sample precipitating out. Sample precipitation can be attributed to poor stability in the starting gradient conditions as paracetamol is soluble in a high percent of organic solvent such as methanol and the method begins with 95% phosphate buffer.

Figure 3: Back pressure is observed at different sample loads on a 90 Å C18, 5 µm 4.6 x 150 mm following the EP 10.7 (Paracetamol and Related Substances) method



In addition to choosing the proper injection volume and mass on column, to further increase column lifetime sample prep should also be considered. Spinning down samples along with filtering them will help protect the column from any particulates. It is also important to be aware of solubility issues with your sample and trying to match the initial mobile phase solvent to the sample solvent. When operating with reversed phase conditions, a column flush at the end of the gradient using a high percentage (≥ 80%) of acetonitrile or methanol will also help rinse any sample or highly retained materials still remaining on the column as well as flushing the LC lines. Additionally, a recommended practice is to utilize a guard column. Guard columns protect the analytical column from particulates and are easy to replace. More information regarding guard columns can be found in the link below.

UTH-SmallMolecule_02_021.pdf (halocolumns.com)



CONCLUSIONS

In conclusion, following the revised EP 10.7 method as written has proved challenging due to the noted increase in back pressure which arises after fewer than normal injections. In our study of these reported issues, it was found this can be attributed to overloading of sample. The concentration should ideally be reduced to 0.125 mg on column vs. 500 µg as the method is written.

REFERENCE

[1] How to Avoid HPLC Column Overload: https://www.chromatographytoday.com/news/hplc-uhplc/31/breaking-news/how-to-avoid-hplc-column-overload/31536

[2] Introduction to Modern Liquid Chromatography, 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan; p. 69-73, 2010, John Wiley & Sons, Inc.

[3] European Pharmacopoeia Supplement 10.7: European Pharmacopoeia Supplement 10.7 now available | EDQM - European Directorate for the Quality of Medicines