Investigation of High Back Pressure on HPLC

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Abstract

At the start of my research project "HPLC Detection of Xanthohumol in Hop Extracts", an HPLC back pressure error appeared. This error indicated that the instrument may have a plug in the capillary tubing or replacement of the HPLC column was necessary. Upon manually cleaning the tubing and replacing the column, the back pressure error did not cease. It was suspected that the pulse dampener was malfunctioning which is yet to be investigated. In conclusion, the source of the high pressure error remains unknown.

Introduction

High Performance Liquid Chromatography (HPLC) is a separation technique. HPLC operates on the same principles as solvent/solvent extraction methods; that is, the analyte must equilibrate between two phases. In the case of HPLC, these phases include a stationary phase (HPLC column) and a mobile phase (solvent). The most important variables that influence the HPLC separation is the composition of the mobile phase and the HPLC column. These variables are responsible for the distribution of the electron cloud (i.e., polarizability) of the analyte. Physically, polarizability influences the analytes' ability to bond to the stationary phase and mobile phase.

In analytical chemistry, methodology is key. The method is a sequence of steps on how to measure any analyte such that the measurement is reproduceable. An HPLC methodology typically includes the history of the analyte, which should address the type of molecule and analytical technique, preliminary analysis, which can help to access the working condition of the instrument, experimental procedure; optimizing procedure, and method validation, which is a statistical analysis of the data that can determine the experimental reproducibility.

One of the reasons why HPLC is a highly reproducible technique is because the pressure is approximately uniform throughout the entire instrument. Unfortunately, the laboratory HPLC encountered a back pressure error. This, physically, means that there is pressure build up somewhere in the system. The possible sources of this error could have been the following: plugged tubing, plugged HPLC column, compromised frits, and/or the pulse dampener.

Conclusion

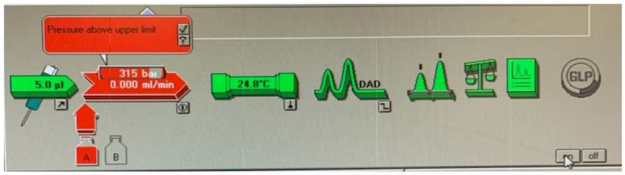
The cause of the back pressure error is currently unknown. Further. investigation will include replacement of the pulse dampener and the recleaning of all tubing in the system. Additionally, if the problem is not resolved with these additional steps, then a trained technician will be required to investigate and validate all technical hardware or software issues

References

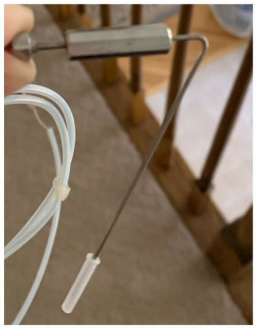
Introduction to Modern Liquid Chromatography 3rd Edition by Lloyd R. Snyder (Author), Joseph J. Kirkland (Author), John W. Dolan (Author), A John Wiley & Sons, Inc., Publication, 2010.

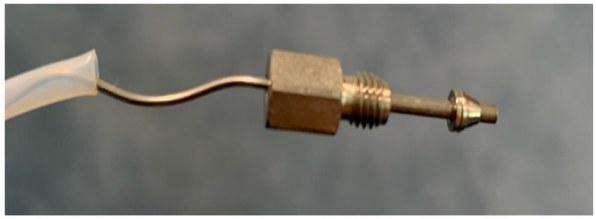
Troubling Shooting Analysis

Instrument diagnosis the for problem as shown in figure.1 below.



However, it was suspected that the back pressure build up was a compounded error. This meant that there may have been multiple locations that were creating a pressure increase. The first and easiest procedure to correct a pressure build-up is to clean out the tubing from the HPLC column to the detector, and the tubing leading to the injector. Interestingly, the stainless steel frit that was connected to the injector snapped for some unknown reason – being stainless steel this should not have happen. The broken frit is shown in figure 2a (left side) and the intact frit is shown in figure 2b (right side).





The tubing in where the pumps are located were not cleaned. It was suspected that the pulse dampener was malfunction - this yet is to be determined. The pulse dampener is shown below