Application Note: SSL2449

HPLC Interferences: Causes and Cures

We have done extensive testing of our products, ensuring that they are capable of producing water suitable for the most demanding requirements of our customers. One criterion was to produce high purity water that can be used with confidence by researchers and analysts performing reverse phase HPLC. We paid close attention to mobile phase interferences and the elimination of ghost peaks that can plague chromatography procedures.

Introduction

In the development stage of our products, we have evaluated the components utilized in these systems ensuring each individual component is capable of producing water meeting our exacting standards. We have compared our results to competitive water systems and performed a direct HPLC comparison utilizing different sources of water, including bottled (HPLC) water.

It is the purpose of this bulletin to report these results, and provide you the techniques we have effectively used to eliminate potential background interferences.

Mobile phase baseline interferences have generally not been a problem at the higher wavelengths, such as 254 nm or above. Most of the interfering organic species are invisible to the detector at these settings. However, interferences or ghost peaks have presented problems at the lower wavelengths such as 210 nm. The following discussion will attempt to address the causes or sources of some of the interferences which may be encountered during reverse phase gradient HPLC procedures which utilize reagent grade water and acetonitrile mobile phase solvents. The following chromatographic method was utilized extensively in the evaluation of both the Thermo Scientific Barnstead Nanopure® UV and Thermo Scientific Barnstead Easypure® UV water systems.

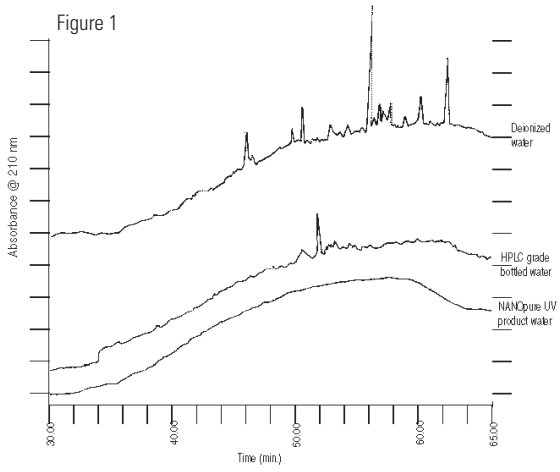
Chromatography Method

60 ml of test water is pumped through a 5 micron C18 ODS 4.6 x 250 mm column with C18 guard column at 2.0 ml/minute for 30 minutes. This is immediately followed by a reverse phase gradient from the 100% water enrichment to 100% acetonitrile at 2.0 ml/minute for 30 minutes. Dual wavelength detection at 254 nm and 210 nm are used during the procedure at 0.05 AUFS. In order for the sample to pass lab evaluation, there must be no peaks greater than 0.0005 AU at 254 nm and 0.001 AU at 210 nm. (The 0.001 AU maximum peak at 210 nm is often limited by the acetonitrile solvent iself). Typical chromatograms will be shown throughout this bulletin.

Chromatographic Comparisons

Figure 1 shows a chromatographic comparison of Nanopure UV reverse phase gradients with bottled HPLC and conventional DI water. Note that both the Nanopure UV and Easypure UV systems produce baselines that are free of ghost peaks that could potentially interfere with your chromatograms.

Figure 1



Determined with a Beckman HPLC system; 165 detector at 210 nm, 4.6 x 250 mm C-18 column; linear gradient mobile phase from 100% water to 100% acetonitrile at 2 mls/minute in 30 minutes, 60 ml sample enrichment at 2 mls/minute. Direct-Connecte™ Guard column.

Sources of Contamination

Elimination of organic contamination begins with the choice of equipment manufacturers. The water system should be constructed of a natural homopolymer (such as polypropylene) with no fillers, plasticizers or mold release agents. Purification media and resins should be semiconductor grade quality. Properly designed adsorption and ultraviolet oxidation techniques should be incorporated into the design of the unit. In addition to these prerequisites, the following potential sources of contamination are presented for consideration.

Final Filter Design

One of our main concerns was the performance of the 0.2 micron absolute filter, used as the final purification process in all reagent grade water systems. Our challenge was to provide absolute filtration without contamination caused by filtration particles, wetting agents, and bubble point chemicals. The amount of water required to rinse a filter, both new and before each use, was evaluated and compared to other filter material available. Figure 2 and Figure 3 show the baselines from a Nanopure UV reagent grade water system utilizing both our hollow fiber filter and a “comparative” filter used on a different reagent grade water system. Figure 2 shows a comparison of the first samples drawn without any appreciable rinse up. Notice the presence of interfering peaks on both filters.

Figure 3 shows the baselines after an approximately 8 liter

Figure 2

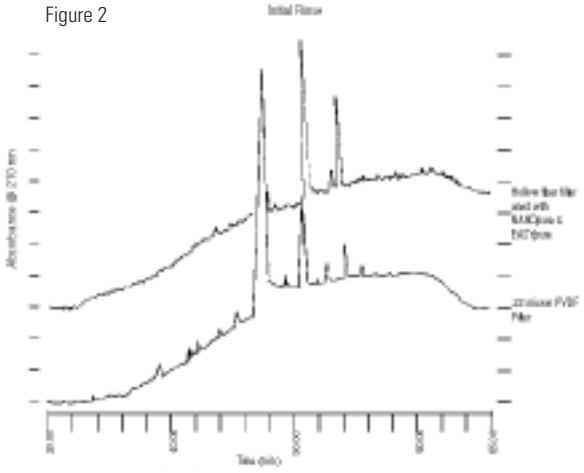
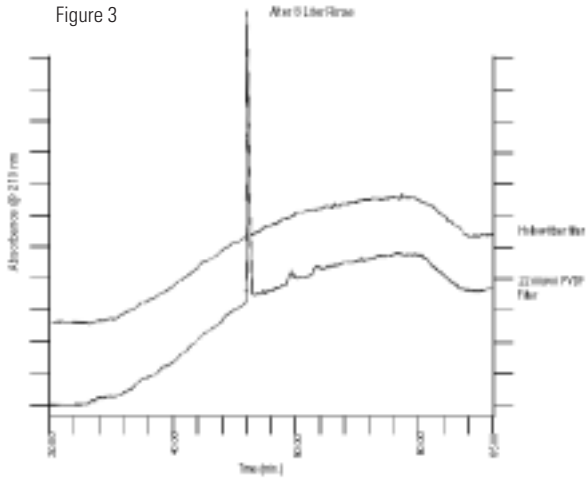


Figure 3



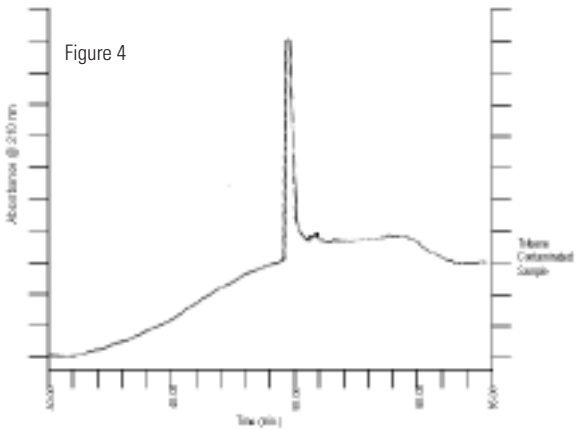
initial rinse. The hollow fiber filter nicely rinsed to a flat baseline, while the other filter still showed substantial peaks. It was repeated after a 16 liter rinse on the other filter material; the peaks were still present.

The conclusion of these assays showed us that the hollow fiber filtration material utilized on our Nanopure and Easypure water purification systems was superior in its ability to rinse out any interfering substances very quickly. We attribute this to the naturally hydrophilic nature of the filtration material, as well as the material utilized for the filter housing. It is also evident that the competitor's filter material required extensive rinse-up to produce anything close to a flat baseline. Requiring such large quantities of water to rinse the 0.2 micron filter (to drain) wastes the capacity of the ion exchange cartridges unnecessarily. It may also take several runs or a column cleaning to remove filter contaminants. This can waste time and solvent.

Laboratory Environment

Peaks in a chromatogram may not be due to contamination in the water system or other mobile phase solvents; organic solvents present in the laboratory environment can cause airborne contaminantion. If organic solvents are handled in the laboratory, the results can and most likely, will be affected. Figure 4 shows the effect of a partially opened container of toluene next to the chromatograph. The chances of environmental contamination affecting a point-of-use water purification system is less because the water is used as it is produced (not opened and closed like HPLC grade bottled water)

Figure 4



Other Contamination Control Considerations

It is necessary to look at other factors when conducting HPLC. Those factors include the baking of glassware to remove any organic contaminants that may have adhered to the glass. Also, the proper purging of a system; at a minimum, the first chromatogram of the day should be thrown away. This will help flush any material out of the chromatograph that may be present from prior samples or that may leak out of the liquid handling hardware of the chromatograph during idle periods. The choice of solvents is extremely important because if the solvent is not pure enough, interferences will exist as a result of using this solvent. We also believe that prompt testing of the samples is necessary. This is required because water of this quality tends to pick up contaminants in a relatively short amount of time.

Summary

The following criteria should be applied to your HPLC determinations to eliminate unwanted interferences.

1) Produce high purity water at the “point-of-use”, with no storage.
2) Utilize a high purity water system which incorporates pure plastic components, semiconductor grade purification media for adsorption and ion exchange, ultraviolet oxidation and properly designed filters to ensure the lowest possible organic effluent.
3) Rinse system and sample prep filter prior to each use.
4) Use good laboratory practices.

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

North America:

USA / Canada
+1 866 984 3766

Europe:

Austria
+43 1 801 40 0

Belgium
+32 2 482 30 30

France
+33 2 2803 2000

Germany national toll free
08001-536 376

Germany international
+49 6184 90 6940

Italy
+39 02 02 95059 434-254

Netherlands
+31 76 571 4440

Nordic countries
+358 9 329 100

Russia / CIS
+7 (812) 703 42 15

Spain / Portugal
+34 93 223 09 18

Switzerland
+41 44 454 12 12

UK / Ireland
+44 870 609 9203

Asia:

China
+86 21 6865 4588 or
+86 10 8419 3588

India
+91 22 6716 2200

Japan
+81 45 453 9220

Other Asian countries
+852 2885 4613

Countries not listed:
+49 6184 90 6940 or
+33 2 2803 2000

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