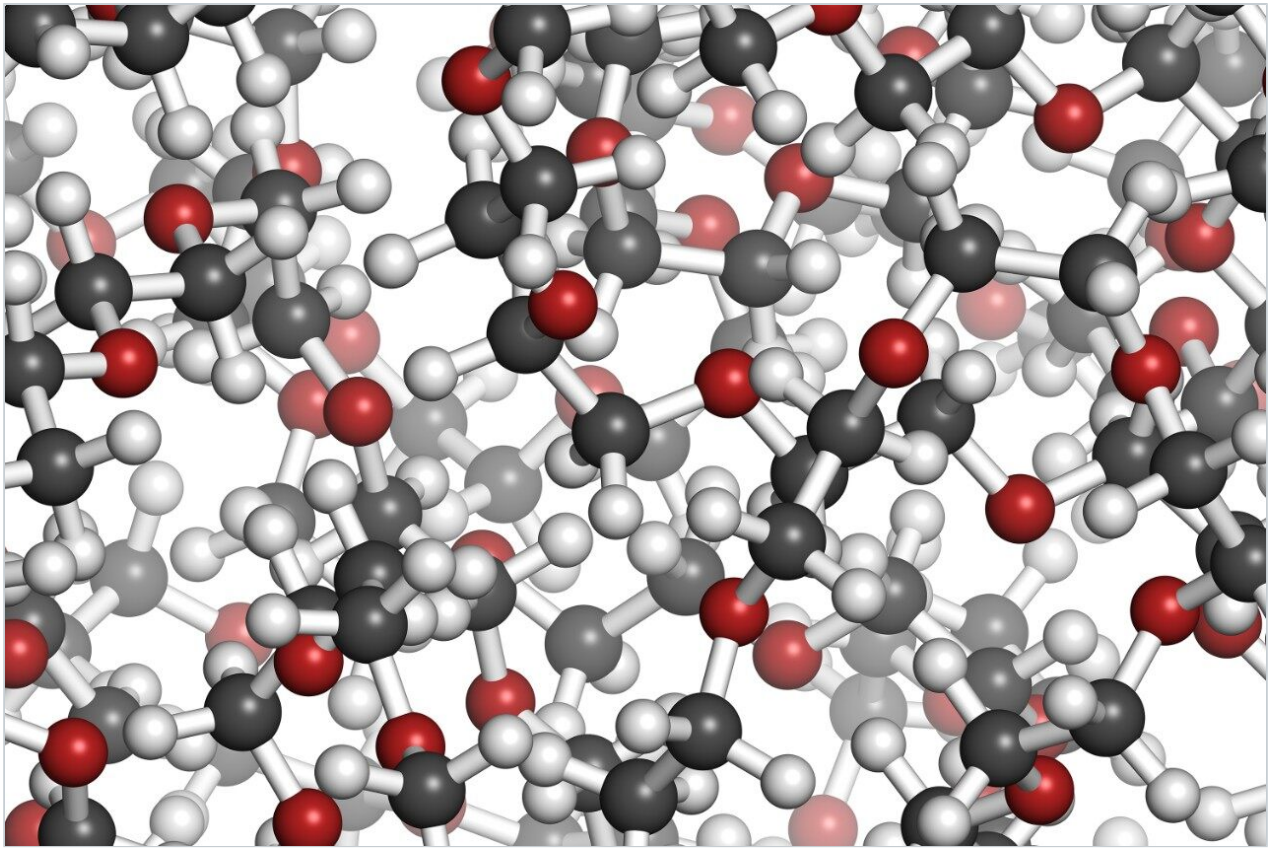
Waters™

Application Note

Confirmation that Only Minimal Levels (≤ 4 pg) of Polyethylene Glycol (PEG) are Present in Waters Nano-LC Consumables

Moon Chul Jung

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This Application brief demonstrates Confirmation of effectiveness of controls during manufacturing to maintain PEG content at minimal levels (≤ 4 pg) in Waters nano-LC consumables.

Benefits

Waters nano columns and traps are free from meaningful amounts of PEG.

Introduction

Polyethylene glycol (PEG) is used in a wide range of applications in chemistry, biology, and medicine. However, the presence of unwanted PEG, which can be introduced through a variety of sources, has the potential to impact proteomic and other nano LC-MS separations. Verification that manufacturing controls employed by Waters ensure only minimal levels of PEG are present in Waters nano-LC consumables is an important reassurance for the users of these products.

Results and Discussion

Waters ACQUITY UPLC M-Class Symmetry C_{18} Trap Columns, 100 Å, 5 µm, 180 µm x 20 mm, 2G, V/M (p/n: 186007496) were evaluated for the level of PEG present post manufacturing. We tested the traps ('Subject Trap') using the customized system consisting of an ACQUITY UPLC M-Class System in conjunction with a Xevo G2-XS QTof Mass Spectrometer (Figure 3). Briefly it was a modified trap-and-elute setup that allowed downstream trapping and analysis of PEG flushed from a test subject. The trap-and-elute setup consisted of an ACQUITY UPLC M-Class HSS T3 Column, 1.8 µm, 75 µm x 150 mm (p/n: 186007473), marked as 'Test Column', and an ACQUITY UPLC M-Class Symmetry C_{18} Trap Column, 100 Å, 5 µm, 180 µm x 20 mm, 2G, V/M (p/n 186007496), marked as 'Test Trap'. A Waters Universal NanoFlow Sprayer MS Source with pre-cut PicoTip Emitters (p/n: 186003916) interfaced the ACQUITY UPLC M-Class System and the Xevo G2-XS QTof MS. PEG standards, used during the method development, and internal standards were prepared using PEG and Leucine Enkephalin (LeuEnk) from the Q-Tof Standards Kit without Bovine (p/n: 700004768). 4 µL of the

internal standard was introduced via partial loop injection from a 5 µL loop. The acetonitrile (ACN), water, and formic acid (FA) used were Optima LC-MS grade supplied by Fisher Scientific.

PEG from either the mobile phases or LC components (system PEG) could potentially impact the results leading to false positive results. A Symmetry C_{18} Column, 100 Å, 3.5 µm, 2.1 mm x 150 mm (p/n: WAT16005), placed on Auxiliary Solvent Manager (ASM) Line A, served as a scrubber column to prevent system PEG from entering the trap-and-elute setup. As the scrubber column can become saturated with PEG, it is important that the column is regularly purged with acetonitrile to remove any collected PEG (22 minutes @ 0.5 mL/min flowrate). It is reconditioned by purging with water (240 minutes @ 49.5 µL/min flowrate) before reconnecting it to the test system.

Initial work using the Micro Binary Solvent Manager (µBSM) and the Micro Sample Manager – Fixed Loop (µSM-FL) demonstrated that preparation of a calibration curve was possible using PEG standards of known concentration, see Figure 2. This method was sensitive to PEG concentrations as low as 1 ppb from 4 µL injections, or 4 pg.

Using this test system setup in Figures a and 3b and the method shown in Table 1, it was possible to flush PEG from the test subject (a trap column) and simultaneously quantify it using the downstream trap-and-elute setup. At the start of each run, the Subject Trap was flushed with 10:90 water/ACN at 0.5 µL/min (from ASM line B) to release any PEG in the trap (Figure 3a). The highly organic flush was mixed with water at 49.5 µL/min (from ASM line A) to bring down the ACN concentration to below 1%, assisting any PEG in the flush to be captured at the Test Trap. The flush/trap process lasted for 10 minutes, which was experimentally determined to ensure complete recovery of PEG. It was also extremely important to accurately control the valve switching time and the ratio of the flow rates from two ASM lines in order to capture PEG in the Test Trap. At the end of the flush/trap step, the TVM valves switched the flow path to isolate the Subject Trap and put the Test Column online (Figure 3b). A gradient flow at 0.5 µL/min was supplied from µBSM to start the separation.

As verification, we dosed the subject trap column with known amounts of PEG and quantitated the PEG using the above described test setup and the method. As the presence of system PEG could potentially impact results, it was important to run blank samples and correct any results for system PEG.

The developed method was applied to test manufactured trap columns. The results shown in Figure 4 are the average of two runs corrected for any system PEG.

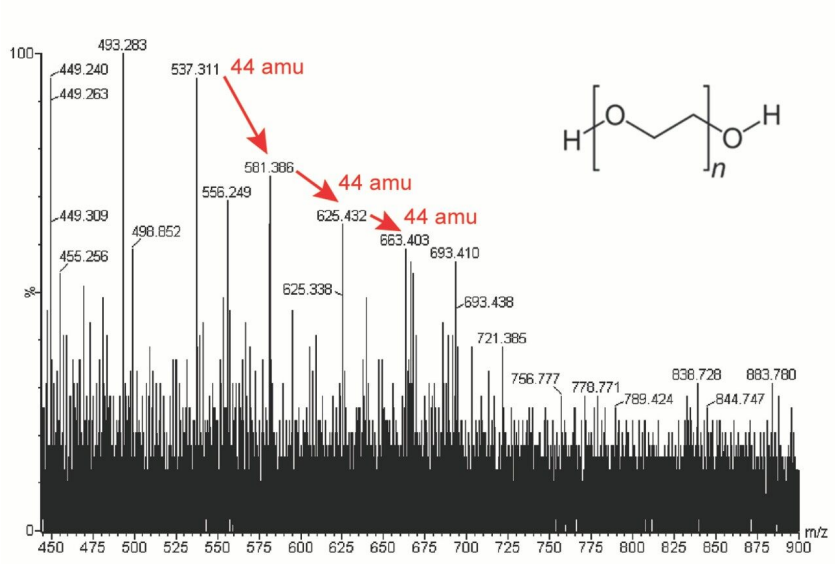


Figure 1. A sample MS spectra showing PEG from a contaminated LC-MS system. Note the 44 amu spacing between the peaks which is characteristic of PEG. The inset shows the general molecular formula of PEG.

PEG standard (pg)	Peak height
0	0
2	9.5
4	285
6	1028
8	2355
10	2328.5
12	3099
16	3201
20	4893

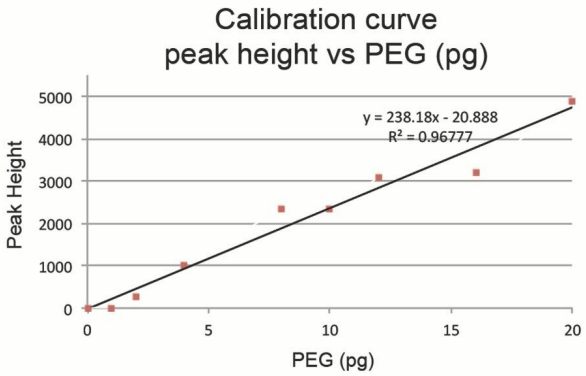


Figure 2. Calibration curve prepared using a simplified setup consisting of a μBSM and μSM-FL. Injection volume was 4 μL.

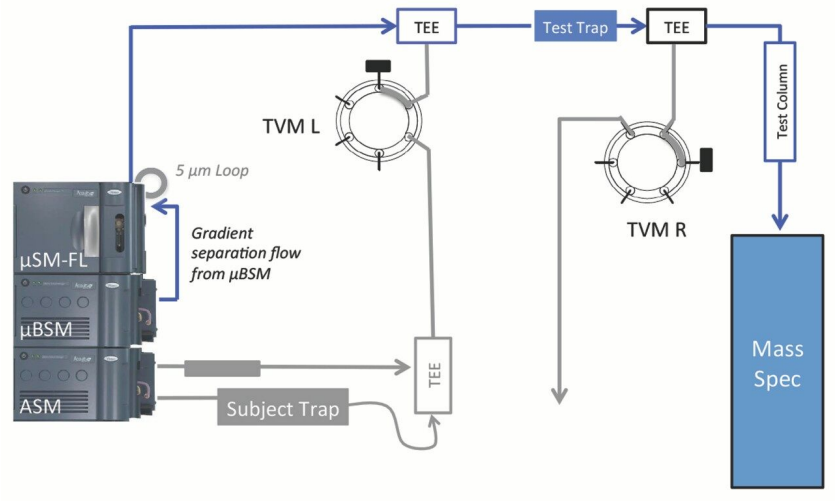


Figure 3a. Test system setup during the flush/trap step. The blue lines denote the active flow path.

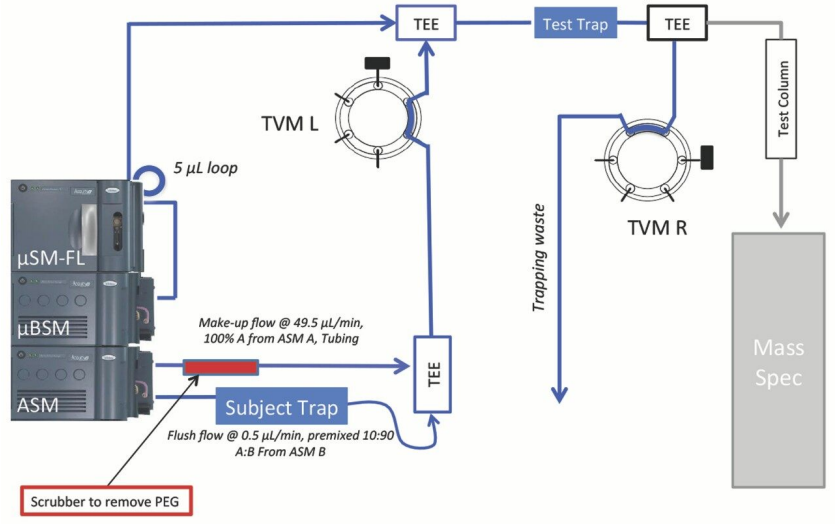
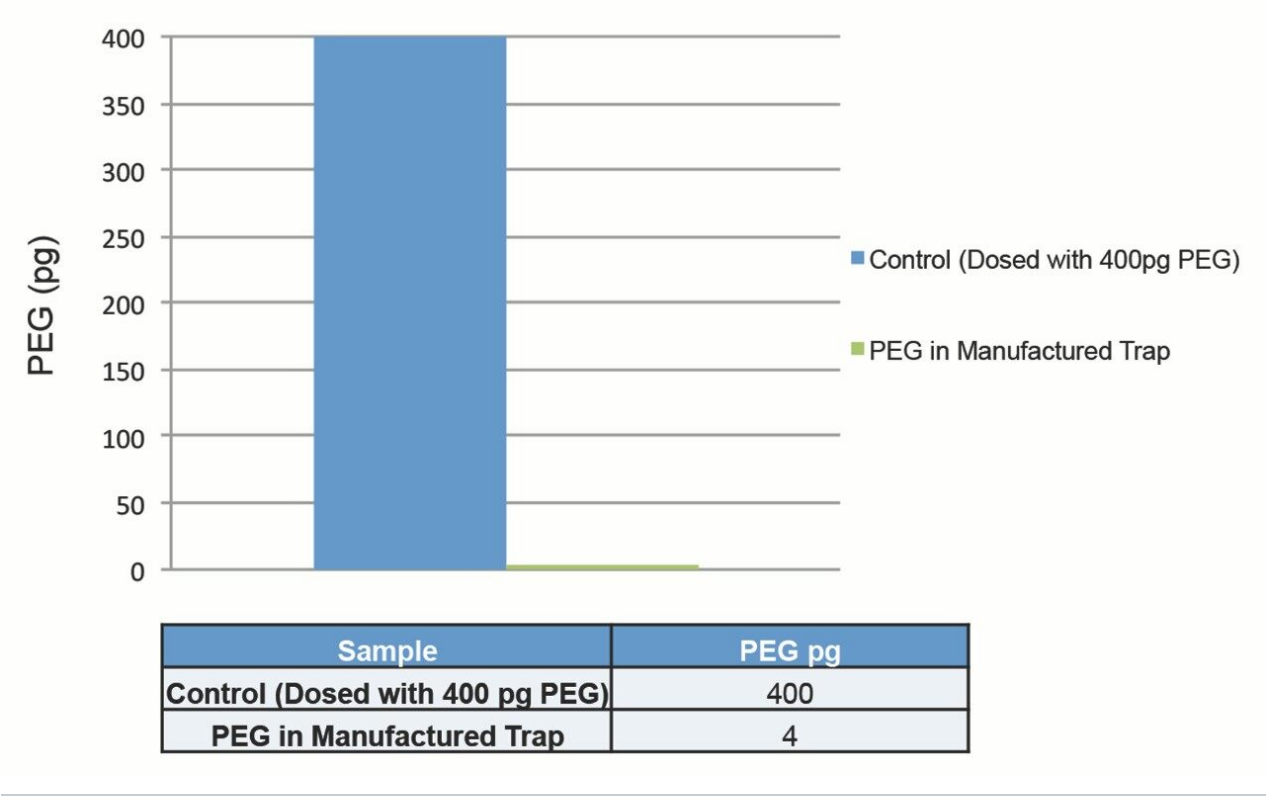


Figure 3b. Test system setup during the elute and analysis step. The blue lines denote the active flow path.

µ BSM	ASM line A (water)	ASM line B (10:90	TVM position

						water/ACN)			
Mode	Time (min)	Flow rate (µL/min)	Water/ACN	Time (min)	Flow rate (µL/min)	Time (min)	Flow rate (µL/min)	L	R
Flush/trap	0	10	99/1	0	70	0	0	1	2
Flush/trap	3	0.5	99/1	0.5	49.5	2	0	1	2
Flush/trap	3.1	0.5	99/1	0.8	49.5	2.2	0.5	1	2
Flush/trap	10	0.5	99/1	10	49.5	10	0.5	1	2
Analysis	0	0.5	99/1	0	5	0	0.5	2	1
Analysis	0.5	0.5	99/1	0.5	5	0.5	0.5	2	1
Analysis	11	0.5	10/90	5	5	5	0.5	2	1
Analysis	18	0.5	10/90	18	5	18	0.5	2	1
Analysis	20	0.5	99/1	20	5	20	0.5	2	1
Analysis	36	0.5	99/1	36	5	36	0.5	2	1
Analysis	36.1	0.5	99/1	36.1	5	36.1	0.05	2	1
Analysis	40	0.5	99/1	40	5	40	0.05	2	1

Table 1. Method for quantifying PEG on subject trap column.



Sample	PEG pg
Control (Dosed with 400 pg PEG)	400
PEG in Manufactured Trap	4

Figure 4. PEG content of manufactured traps in comparison to control (dosed with 400 pg PEG).

Conclusion

This study confirms the effectiveness of controls during manufacturing to produce trap columns with minimal levels of PEG (≤ 4 pg). We developed a method to quantify the PEG content of nano-LC trap columns using a modified trap-and-elute setup. A key aspect of the method is the use of a scrubber column to reduce the amount of PEG present in the LC system. The method developed to detect and measure PEG present in nano-LC traps is sensitive to 4 pg.

Featured Products

ACQUITY UPLC M-Class System <https://www.waters.com/134776759>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

720006427, December 2018