WATERS PAH COLUMN

I. INTRODUCTION

We are sure you will find that Waters built-in quality helps solve many of your challenging separation problems. We strive to provide products with the highest degree of lot-to-lot and column-to-column reproducibility to minimize variations in your chromatographic results. Waters PAH columns are manufactured and packed under highly controlled conditions. Each must pass a series of stringent tests before being accepted for shipment. Included with each column is the final Certificate of Analysis.

Polynuclear Aromatic Hydrocarbons (PAHs) are among the most frequently monitored environmental contaminants. Standard and official methods for the analysis of PAHs are found in compendia for air, drinking water, wastewater, solid waste, and food analysis1.

Many of these methods specify HPLC, usually with UV and fluorescence detection, as the recommended analytical procedure.

Waters PAH columns are optimized for the HPLC analysis of PAHs. Figure 1 shows a chromatogram of 16 PAH compounds, listed as target pollutants by the U.S. EPA. The Waters PAH columns achieve baseline resolution and excellent peak symmetry for all 16 target analytes in less than 25 minutes, while employing a simple water; acetonitrile binary gradient. The resolving power of the PAH Columns provides superior peak identification and quantitation for PAHs.

Florida Administrative Code 17.700 includes 2 additional compounds (1-methyl naphthalene and 2-methyl naphthalene) in addition to the 16 compound EPA 610 mix that we currently use to show the proficiency of Waters instrumentation to analyze PAH compounds. The new Waters PAH columns resolve these two compounds along with the other 16, (see Figure 2).

CONTENTS

Column: Waters PAH Column 5 µm 4.6 x 250 mm @ 27 °C
System: Waters Alliance System with 2996 Photodiode Array Detector
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 60% B to 100% B using curve 9 in 12 minutes, hold 11 minutes, back to initial conditions
Flow Rate: 1.2 mL/min
Injection: 20 µL
Sample: EPA-610 mixture

Peaks:
1. Naphthalene - 20 ppm
2. Acenaphthylene - 40 ppm
3. Acenaphthene - 20 ppm
4. Fluorene - 4 ppm
5. Phenanthrene - 2 ppm
6. Anthracene - 2 ppm
7. Fluoranthene - 4 ppm
8. Pyrene - 2 ppm
9. Benzo(a)anthracene - 2 ppm
10. Chrysene - 2 ppm
11. Benzo(b)fluoranthene - 4 ppm
12. Benzo(k)fluoranthene - 2 ppm
13. Benzo(a)pyrene - 2 ppm
14. Dibenzo(a,h)anthracene - 4 ppm
15. Benzo(g,h,I)perylene - 4 ppm
16. Indeno(1,2,3-cd)pyrene - 2 ppm

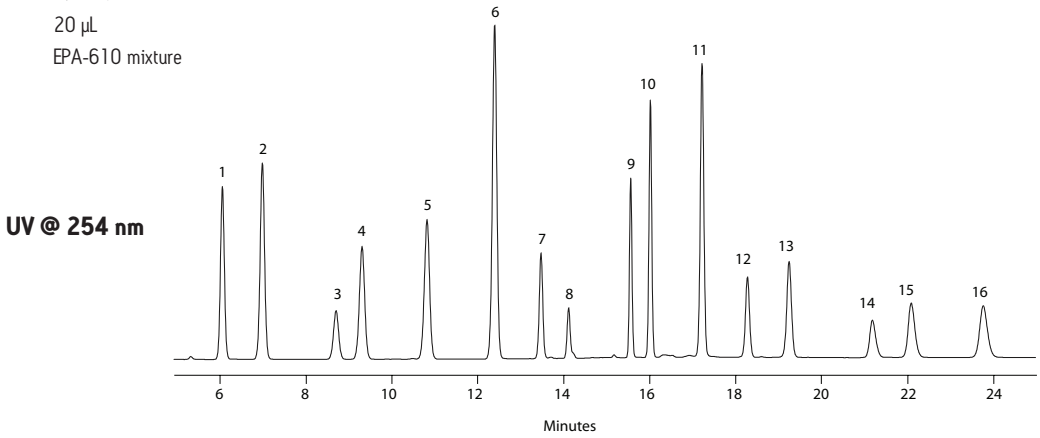

Figure 1: PAH Analysis using Waters PAH Columns

Column: Waters PAH Column 5 µm 4.6 x 250 mm @ 27 °C
Eluent A: Water
Eluent B: Acetonitrile
Gradient: 60% B to 100% B using curve 9 in 12 minutes hold 11 minutes, back to initial conditions
Flow Rate: 1.2 mL/min
Injection: 20 µL
Sample: EPA-610 mixture plus two compounds*

Peaks:
1. Naphthalene - 20 ppm
2. Acenaphthylene - 40 ppm
3*. 1-methyl naphthalene - 25 ppm
4*. 2-methyl naphthalene - 25 ppm
5. Acenaphthene - 20 ppm
6. Fluorene - 4 ppm
7. Phenanthrene - 2 ppm
8. Anthracene - 2 ppm
9. Fluoranthene - 4 ppm
10. Pyrene - 2 ppm
11. Benzo(a)anthracene - 2 ppm
12. Chrysene - 4 ppm
13. Benzo(b)fluoranthene - 4 ppm
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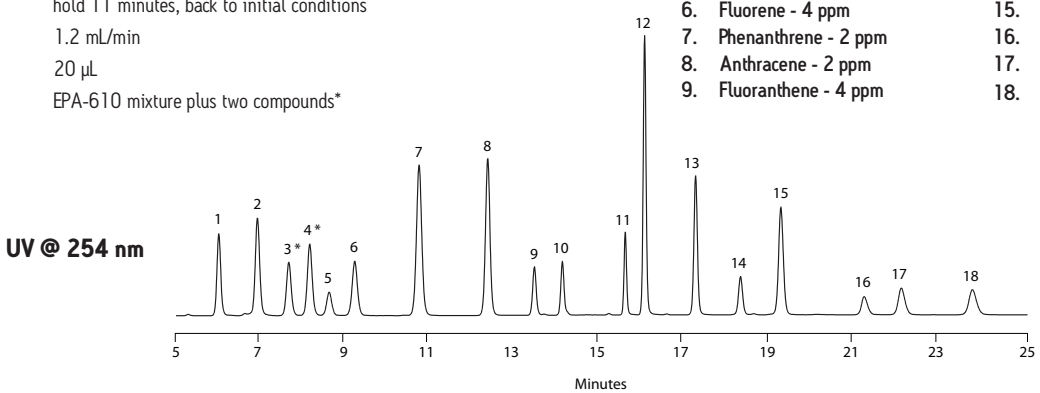


Figure 2: PAH Analysis According to Florida Administrative Code 17,700

II. CONNECTING THE COLUMN TO THE HPLC INSTRUMENT

Handle the column with care. Do not drop or hit column on a hard surface as it may disturb the bed and affect its performance.

1. Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results.
2. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16 inch outer diameter stainless steel tubing. When tightening or loosening the compression screw, place the 5/16 inch wrench on the compression screw and the other 3/8 inch wrench on the hex head of the column endfitting. Note: If one of the wrenches is placed on the column flat during this process, the endfitting will be loosened and leak.

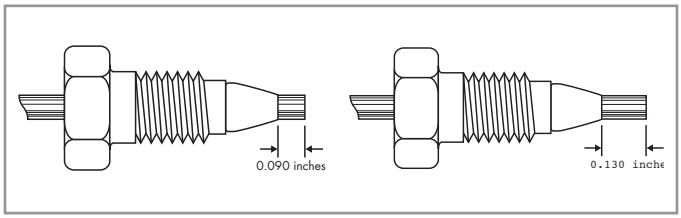
3. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing and ferrule must be assembled.

4. An arrow on the column identification label indicates correct direction of solvent flow.

It is important to realize that extra column peak broadening can destroy successful separation. The choice of appropriate column connectors and system tubing is discussed in detail below.

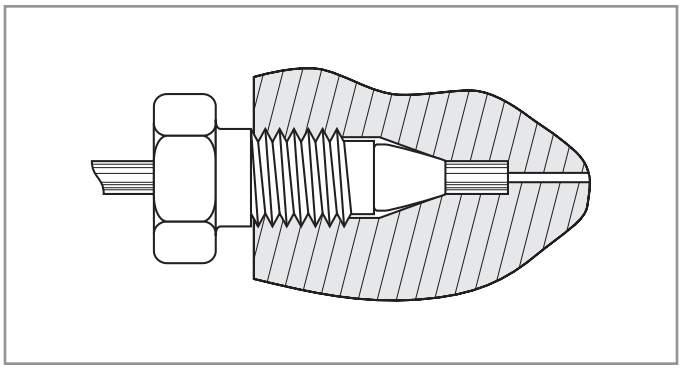
a. Column Connectors and System Tubing Considerations

Due to the absence of an industry standard, various column manufacturers have employed different styles of chromatographic column connectors. The chromatographic performance of your separation can be negatively affected if the style of your column endfittings do not match the existing instrumentation tubing ferrule setting. This page explains the difference between Waters style and Parker style endfittings, which vary in the required length of the tubing protruding from the ferrule. The PAH column is equipped with Waters style endfittings which require a 0.130 inch ferrule depth (see next section for setting ferrule depth). If you are presently using a non-Waters style column, it is critical that you reset the ferrule depth for optimal performance.



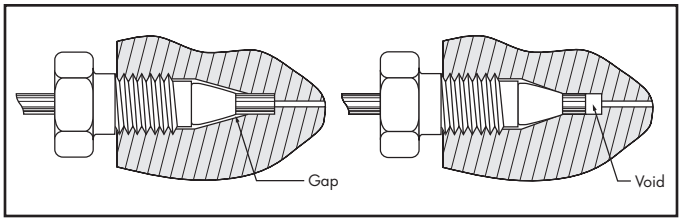
The Proper Tubing/Column Connection

Tubing touches the bottom of the column endfitting, with no void between them.



A void appears if a tube with Parker ferrule setting is connected to a Waters style column.

The presence of a void in the flow stream downgrades the column performance. There is only one way to fix the problem: Cut the end of the tubing with the ferrule, put a new ferrule on the tubing and make the connection. Before tightening the screw, make sure that the tubing bottoms out in the endfitting of the column.



If tubing with a Waters style ferrule setting is connected to a column with Parker style endfitting, the end of the tubing will bottom out before the ferrule reaches its proper sealing position. This will leave a gap creating a leak. There are two ways to fix the problem:

1. Just tighten the screw a little bit more. The ferrule moves forward, and reaches the sealing surface. Do not overtighten because this may end in breaking the screw.

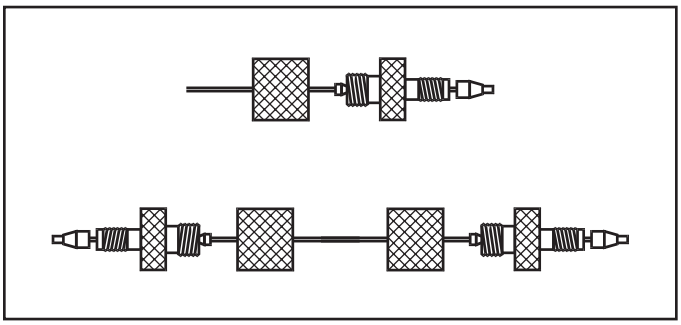
2. Cut the tubing, put a new ferrule on it and make the connection.

An alternative is to replace the conventional compression screw fitting with an all-in-one PEEKTM fitting.

(Waters part number PSL613315) that allows you to reset the ferrule depth. Another approach is to use a SLIPFREE® fitting to always ensure the correct fit. The finger-tight SLIPFREE connectors automatically adjust to fit all compression screw type fittings without the use of tools.

b. SLIPFREE Connectors

Guarantees a void-free connection because it pushes the tubing into the endfitting; This design comes installed on the tubing. Fingertight to 10,000 psi – never needs wrenches. Readjusts to all column endfittings. Compatible with all commercially available endfittings. Unique design separates tube-holding function from sealing function.



c. Minimization of Bandspreading

The following figure shows the influence of tubing internal diameter on system band spreading and peak shape. As can be seen, the larger tubing diameter causes excessive peak broadening and lower sensitivity.

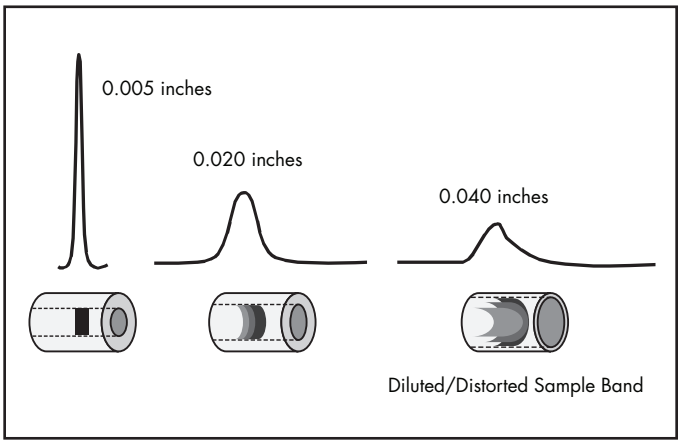


Figure 3: Effect of Connecting Tubing on System

d. Measuring System Bandspread Volume

1. Disconnect column from system and replace with a zero dead volume union.
2. Flow rate 1 mL/min. This should be performed on a single wavelength detector (not a PDA/DAD).
3. Dilute a test mix in mobile phase to give a detector sensitivity 0.5-1.0 AUFS (can use the system start up test mix which contains uracil, ethyl and propyl parabens; Waters part number WAT034544).
4. Inject 2 to 5 mL of this solution.

In a typical LC bandspreading volume system should be 100 mL ± 30 mL (or variance of 400 µL2 +/- 36 µL2)

Microbore (2.1 mm i.d. and smaller) system should be no greater than 20-40 mL

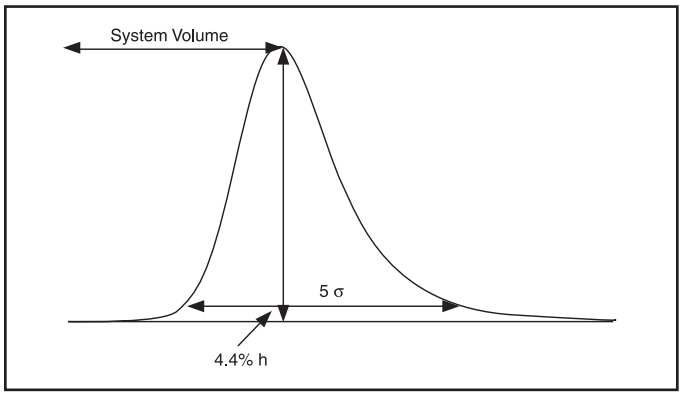


Figure 4: Determination of System Bandspread Volume using the 5-Sigma Method

e. Measuring Gradient Delay Volume

1. Replace the column with a zero dead volume union.
2. Determine the gradient-delay or dwell volume for your system by performing the following test. Prepare eluent A (pure solvent, such as methanol) and eluent B (solvent plus sample, such as 5.6 mg/mL propylparaben in methanol).
3. Equilibrate the system with eluent A until a stable baseline is achieved. Switch to 100% eluent B and record the half height of the step. Refer to Figure 5 for an illustration.

The dwell volume should be less than 1 mL. If this is not the case, see section on System Modifications (below) to reduce your system volume.

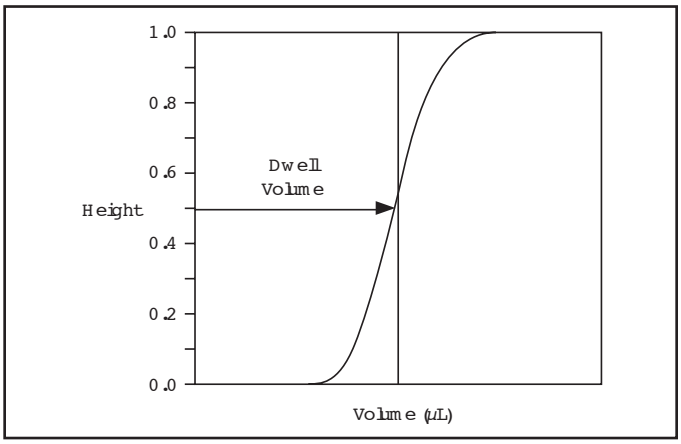


Figure 5: Determination of Dwell Volume

f. Use of Narrow-Bore Columns – (3.0 mm i.d.)

This section describes how to minimize extra column effects and gives some guidelines on how to maximize the advantages of your narrow-bore column. The 3.0 mm i.d. narrow-bore column usually requires no system modifications. With the 2.1 mm i.d. column, however, modifications to your HPLC system may be required in order to eliminate excessive system bandspread volume. Without proper system modifications, excessive system bandspread volume causes peak broadening and has a large impact on peak width as peak volume decreases.

g. Impact of Bandspreading on Column Performance (2.1 mm i.d. column)

System with 70 mL bandspread >> 10,000 plates
System with 130 mL bandspread >> ~8,000 plates (same column)

Note: Flow splitters after the column will introduce additional bandspreading.

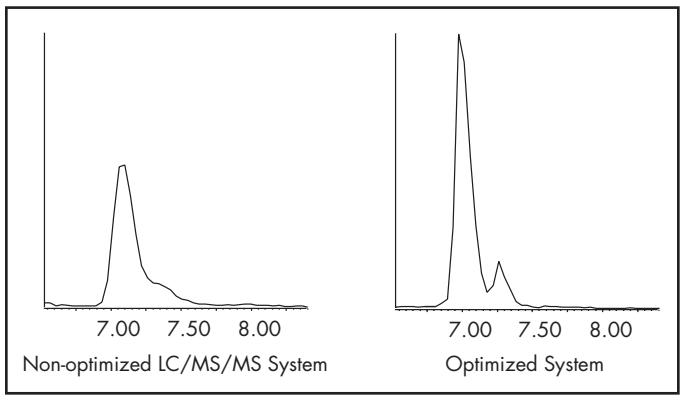


Figure 6: Impact of Bandspreading

Optimizing a system, especially one using flow splitters can have a dramatic effect on sensitivity andresolution. Use of correct ferrule depth connectors and minimizing tubing diameter and lengths showed a doubling of sensitivity and enabled resolution of the metabolite on this LC/MS/MS system.

h. System Modification Guidelines

1. Use a microbore detector flow cell with the 2.1 mm columns. Recall that due to the shorter pathlength, detector sensitivity is reduced to achieve lower band spread volume.
2. Injector sample loop should be reduced to minimum.
3. Use 0.009 inch (0.25 mm) tubing between pump and injector.
4. Use 0.009 inch (0.25 mm) tubing for rest of connections in standardsystems and 0.005 inch (0.12 mm) tubing for narrowbore (2.1 mm i.d.) systems.
5. Use perfect (pre-cut) connections (with a variable depth inlet if using columns from different suppliers).
6. Time constants should be shortened <0.2 Column Equilibration.

III. COLUMN EQUILIBRATION

Waters delivers the column in 100% acetonitrile. It is important to ensure solvent compatibility before changing to a new solvent. Equilibrate your column with a minimum of 10 times its internal volume with the mobile phase to be used (refer to Table 1 for some standard column volumes).

1. Purge your pumping system and then connect the inlet end of the column to the injector outlet. Turn on the pump flow at 0.1 mL/min. and increase to 1 mL/min over 5 minutes.
2. When the solvent is flowing freely from the column outlet, attach the column to the detector. This procedure prevents entry of air into the detection system and gives more rapid equilibration.
3. When the mobile phase is changed, gradually increase the flow rate of the new mobile phase from zero mL/min to 1.0 mL/min in 0.1 mL/min increments.
4. Once a steady backpressure and baseline have been achieved, the column is ready to be used.

Note: If mobile phase additives are present in low concentrations (such as ion-pairing reagents, at 5 to 10 mmol/L) 100 to 200 column volumes may be required for complete equilibration.

Table 1. Volume of Standard Column (mL), Multiply by 10 for Flush Solvent Volume

	Column Internal Diameter (mm)		
Column Length	2.1	3.0	4.6
50 mm	0.2	0.3	0.8
100 mm	0.4	0.7	1.7
150 mm	0.5	1.0	2.5
250 mm	0.9	1.8	4

IV. COLUMN USAGE

To ensure the continued high performance of your columns and cartridges, follow these guidelines:

a. Guard Columns

Sample impurities very often contribute to column contamination.
Two ways to avoid this are:

a. Use of Waters Oasis® solid-phase extraction sample clean-up cartridges or columns or Sep-Pak® cartridges of the appropriate chemistry to clean up your sample before analysis.

b. Use of a Waters guard cartridge of matching chemistry and particle size between the injector and main column. It is important to use a highperformance matching guard column to protect the main column while not compromising analytical resolution.

b. pH Range

Recommended pH ranges for solvent and buffer combinations for Waters PAH columns are between 2.0 and 8.0. A pH less than 2 may cause hydrolysis of the bonded phase. At a pH greater than 7.0, the alkaline solvent buffers will attack the silica substrate resulting in void formation in the column as the silica solubilizes.

c. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all buffers before use. Pall Gelman Laboratory Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poorer performance. Degas all solvents thoroughly before use to prevent bubble formation in the pump and detector.

d. Pressure

All Waters PAH columns, regardless of dimension, can be operated at pressures up to 6000 psi, 400 bar or 40 Mpa.

e. Temperature

Temperatures between 20 – 50 °C are recommended for operating Waters PAH columns to enhance selectivity, lower solvent viscosity and increase mass transfer rates. However, any temperature rise above ambient will have a negative effect on lifetime which will vary depending on the pH and buffer conditions used.

V. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

A shift in retention or resolution may indicate contamination of the column Flushing with a neat organic solvent is usually sufficient to remove the contaminant. If the flushing procedure does not solve the problem, purge the column with a sequence of progressively more nonpolar or hydrophobic solvents. For example, switch from water to tetrahydrofuran (THF) to methylene chloride. Return to the standard mobile phase conditions by reversing the sequence. Guard columns need to be replaced at regular intervals as determined by sample contamination. When system backpressure steadily increases above a set pressure limit, it is usually an indication that the guard column should be replaced.

b. Storage

For periods longer than four days store the column in 100% acetonitrile. Do not store columns in buffered, acidic or basic eluents. If the mobile phase contained a buffer salt flush the column with 10 column volumes of HPLC grade water (see Table 1) and replace with 100% acetonitrile. Completely seal column to avoid evaporation and drying out of the bed.

VI. TROUBLESHOOTING

Changes in retention time, resolution, or backpressure are often due to column contamination. See the Column Cleaning, Regeneration and Storage section of this instruction sheet. Information on colum troubleshooting problems may be found in HPLC Columns Theory Technology and Practice, U.D. Neue, (Wiley-VCH, 1997) or the Waters HPLC Troubleshooting Guide (Literature code # 720000181EN).

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