Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0163 4/84
DP/LS/MD/AA/DV

PTH AMINO ACIDS III:

SYSTEM CONFIGURATION FOR RAPID GRADIENT ANALYSIS

In a recent Lab Highlight (LAH 0143), a very fast separation of 22 phenylthiohydantoin (PTH) amino acids on a NOVA-PAK™ C_{18} steel column was described. A critical factor in maintaining reproducibility from one chromatographic system to the next is maintaining a constant system plumbing configuration.

The PTH Amino Acid System consisted of the following LC hardware: two M6000A pumps, an M720 controller, an M710B WISP™ autosampler, a column heater and an M440 fixed wavelength detector. M510 pumps work as well as M6000A pumps, and it is possible that the "B" pump could be replaced with an M45 pump. An M680 or 721 controller can be used instead of the M720, and a U6K injector may work if proper precautions are taken (see below). It is very possible that other configurations of Waters™ hardware (e.g. two M45 pumps or an M481 detector) would yield inferior results or decreased performance. This would be especially true for high sensitivity analyses in which gradient-caused disturbances are not minimized in the M481 (remember only the M440, 441, and 490 detectors have the TAPER-CELL™ design.)

Another note of caution is that other manufacturers' equipment may exhibit inferior performance in comparison to Waters™ hardware. While working with a prospective customer, our San Francisco people traced a failure with the NOVA-PAK™ column method to its use in a Spectra-Physics SP8000 system (Figure 1). Obviously, these results are not satisfactory, but the same column in a Waters™ gradient LC system afforded the expected results (See Lab Highlight 0115 for a comparison with the S-P system). Similar problems may be experienced with other low pressure blending systems or systems with large volume dynamic mixers.

Even with the right equipment, it is important to take care to plumb the system correctly. The output of Pump B should be plumbed into the reference valve of Pump A where it mixes with the A eluent. The combined output is routed through the A pump pulse dampener and pressure transducer. A blue line (1 meter of .040" ID tubing) connects the A pump to the WISP™ autosampler. The 200 µl loop must be used in the WISP™ autosampler, not the 2ml loop which is wound around the injector. If a U6K injector is used, the maximum loop size recommended is also 200 µl rather than the standard 2 ml loop. After the injector a red line (1 meter of .009" ID tubing) is connected to an in-line filter which is placed inside the column heater. A short connecting tube (.009" ID) joins the filter and the column which is placed in a heater block. The line from the column to the detector is also .009" ID and should not exceed 1 meter in length.

Waters
Division of MILLIPORE 34 Maple St. / Milford, MA 01757 / 617-478-2000
Steve Cohen

We have seen problems with several systems in which the cause has been traced to the use of alternate plumbing schemes. The most frequent error is the use of a large, 2 ml loop in the WISP™ autosampler or a U6K injector. The extra tubing in these systems significantly increases the gradient-delay volume (the volume in the system between the point of mixing and the head of the column), and a consequent decrease in chromatographic resolution has been observed. No extra switching valves or similar accessories should be placed in the fluid path. Also, there is presently no satisfactory guard column for the system and, therefore, it is recommended that none be used.

I hope this information will be helpful to those in the field who are setting up PTH analysis systems, and remember that much of this advice can be useful for any rapid gradient analysis.

FIGURE 1

Hardware:	Spectra-Physics SP8000
Eluent A:	80% 25mM Sodium Acetate pH 5.10 20% CH_3CN
Eluent B:	25% H_2O 25% 2-propanol 50% Eluent A
Flow Rate:	1 ml/min
Program:	Step from Eluent A to Eluent B at 0.5 minutes
Peak Identification: ?