

Waters

# Lab Highlights

LAH 0229 3/85  
DP/LS/ED/AA/DV

## USING A U6K INJECTOR FOR PICO-TAG™ ANALYSIS

The PICO-TAG™ method of amino acid analysis has proved to be a tremendously advantageous technique to evaluate protein composition (see LAH 0163 4/84). Retention time reproducibility, a critical factor in recognizing peak identities, has been shown to be very high using the M710B WISP™ autosampler, but what if the number of analyses required does not justify the purchase of an autosampler? Can the reliability with a WISP™ (Table 1) be met with a U6K manual injector?

The answer is YES, but there are several modifications that must be followed in order to ensure high quality results. First, the fluid path of the U6K system must be made similar to that of a WISP™ system (Figure 1). A blue line (P/N 72214, 1 meter of .040" ID tubing) is used to connect the U6K with the pump, the standard 2 ml loop is replaced with a 250 µl loop (P/N 96125) and the outlet line from the U6K to the in-line filter is a WISP™ red line (P/N 72215, 1 meter of .009" ID tubing). If all other operating parameters (hardware, flow, gradient, table, temperature) are kept identical to the normal PICO-TAG™ system, the usual excellent resolution will be maintained.

There is one additional factor in retaining high system performance--the volume of re-equilibration solvent. If a timer is used and injections made with the U6K at precise intervals (approximately 21 minutes between injections) reproducibility of retention times will be similar to that of the WISP-based system. This may not be convenient, as this would require close monitoring of the system, so it is important to know the effect of varying the equilibration time. The data in Table 2 illustrate the potential problem. When the time between injections is between 28 and 40 minutes (approximately 18-33 ml of solvent for re-equilibration), there is a slow but steady increase in retention from Ser to Pro, which can be large enough to cause misrecognition of peak identities by the integrator. However, this shift in retention becomes insignificant after 40 minutes (33 ml), so that if untimed injection is desirable, good retention time reproducibility can be achieved if this minimum equilibration volume is used. The bottom line is that a U6K-based PICO-TAG™ system offers a very affordable amino acid analysis system with state-of-the-art performance.

TABLE 1

COEFFICIENT OF VARIATION FOR EIGHT CONSECUTIVE RUNS  
OF 250 PICOLES

	Retention Time %
Asp	0.50
Glu	0.45
Ser	0.41
Gly	0.37
His	0.19
Arg	0.23
Thr	0.13
Ala	0.21
Pro	0.16
Tyr	0.37
Val	0.22
Met	0.22
Cys	0.14
Ile	0.07
Leu	0.08
Phe	0.11
Lys	0.06
Average	0.23

TABLE 2

RETENTION TIME OF PICO-TAG™ DERIVATIVES  
AS A FUNCTION OF EQUILIBRATION DELAY USING A U6K INJECTOR

Time Between Injections	28	34	40	45	52
Amino Acid					
Asp	1.82	1.83	1.85	1.85	1.84
Glu	2.07	2.08	2.10	2.10	2.10
Ser	4.27	4.30	4.33	4.34	4.34
Gly	4.71	4.75	4.77	4.80	4.80
His	5.35	5.40	5.46	5.50	5.50
Arg	5.88	5.94	5.99	6.02	6.03
Thr	6.05	6.10	6.15	6.18	6.18
Ala	6.18	6.24	6.28	6.30	6.30
Pro	6.35	6.41	6.45	6.48	6.48
Tyr	7.61	7.65	7.66	7.68	7.67
Val	8.05	8.07	8.08	8.10	8.09
Met	8.40	8.43	8.44	8.45	8.45
Cys	8.97	8.99	8.99	9.00	8.99
Ile	9.28	9.30	9.30	9.31	9.30
Leu	9.43	9.45	9.45	9.46	9.45
Phe	10.24	10.25	10.25	10.26	10.25
Lys	11.21	11.21	11.22	11.23	11.22

FIGURE 1

