Waters
Lab Highlights
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AN/System Performance

MAXIMIZING SYSTEM PERFORMANCE
EFFECT OF U6K INJECTOR LOOP VOLUMES

We have occasionally heard comments to the effect that maximum U6K injector performance and maximum column efficiencies, especially during plate count testing, can only be obtained if the standard 2 ml injection loop is replaced with a 250 µl loop. This is wrong! Because of the design of the injector, **there is no reason for this to be the case.**

A standard U6K injector is equipped with a 2 ml sample loop but alternate sizes (e.g. 250 µl, etc.) are available. The internal flow configuration for this injector is such that the sample solution is loaded into the injection loop from the outlet end. When the injection is performed, the last microliter of sample solution to be loaded becomes the first to reach the inlet of the column ("last in, first out"). With such a design, column performance in an isocratic separation should be independent of the volume of the U6K injector sample loop as long as it is large enough to load the total sample.

The following data (Table1; Figure 1) is presented to demonstrate that measured column efficiency, even for a very high efficiency Ultrastyragel® column, is independent of the volume of the sample loop in a U6K injector. The plate count of a 10^5Å Ultrastyragel column was measured in duplicate with a system containing a U6K injector equipped with a 2 ml loop (Runs 1A, 1B). The U6K was then equipped with a 250 µl loop and the column plate count was retested (Runs 2A, 2B). The 2 ml loop was replaced and the initial data was repeated (Runs 3A, 3B). The average plate counts are identical (within experimental error) regardless of which loop was used. Accurate plate count measurements can thus be performed without changing to a smaller (e.g. 250 µl) loop.

TABLE 1

Run #	U6K Loop	N_{tan}	$N_{5\sigma}$
1A	2 ml	18,012	17,830
1B	2 ml	18,001	17,210
		Ave 18,007 p/ft	**Ave 17,520 p/ft**
2A	250 µl	18,058	17,641
2B	250 µl	18,200	17,649
		Ave 18,129 p/ft	**Ave 17,645p/ft**
3A	2 ml	18,197	16,786
3B	2 ml	18,185	17,662
		Ave 18,191 p/ft	**Ave 17,224 p/ft**

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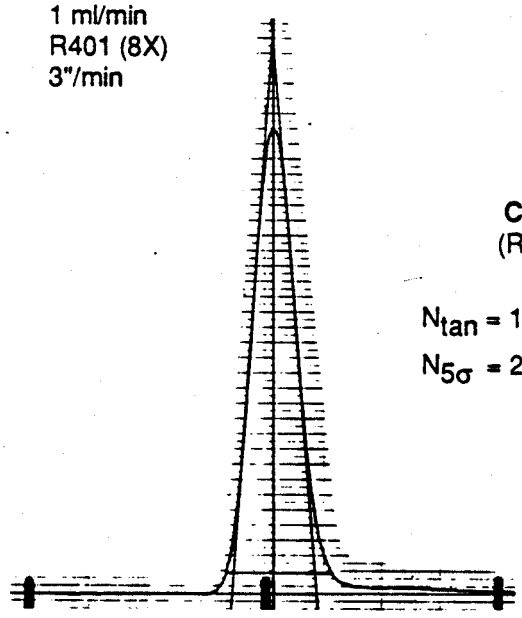
Figure 1

Sample:	o-Dichlorobenzene (ODCB) (3% in Toluene)
Inj'n Vol:	10 µl
Column:	10^5Å Ultrastyragel®
Mobile Phase:	Toluene
Flow Rate:	1 ml/min
Detector:	R401 (8X)
Chart Speed:	3"/min



Chromatogram from Run 1A
(Reduced to 41% of original chart)

$N_{tan} = 16\ (916.0\ mm / 27.3\ mm)^2 = 18{,}012\ p/ft$

$N_{5\sigma} = 25\ (916.0\ mm / 34.3\ mm)^2 = 17{,}830\ p/ft$

Under typical conditions, the standard sample loop (2 ml) can be used without hesitation in an isocratic system. Keep in mind that introduction of the sample loop (at atmospheric pressure) into the high pressure system results in a momentary drop in system pressure and a short (but finite) amount of time will be required to repressurize the system to normal operating pressure. Use of a larger loop would then cause peaks to elute at slightly longer retention times than with a smaller loop. In the case of the data in Table 1, the ODCB peak elutes in 12.02 min with a 2 ml loop (Run 1A) and in 11.99 min with a 250µl loop (Run 2A). The difference is small on the basis of time but is repeatable and easily measured from the recorder chart (Δ = 2.2 mm) at the fast chart speed used for plate count determinations (Figure 1).

In the case of gradient elution, especially where fast analyses such as the 11 min analysis of PTH amino acids [1] are performed, the effect of a larger volume injection loop is to delay onset of the gradient at the head of the column as well as to possibly alter a gradient profile previously optimized with a smaller injection loop. This delayed/altered gradient profile may result in different chromatographic resolution[2] with the larger loop, but this has nothing to do with plates.

Similar considerations would be valid for the WISP™ autosampler which is delivered with a 200 µl sample loop as standard but is also equipped with an auxiliary 2 ml loop that can easily be installed as needed.

1. S. Cohen, Waters Lab Highlight, #0143 (12/83).
2. S. Cohen, Waters Lab Highlight, #0163 (4/84).