

**Maximum Loop Size versus the Number of Injections per Vial
for Alcott Positive Displacement Autosamplers**

Alcott/Micromeritics has manufactured three different models of HPLC autosamplers based on the "Positive Displacement" method of sample transfer. The method incorporates the completely filled loop or Fixed Loop technique of sample injection into the liquid chromatograph. Injection size is adjusted by changing the size of the sample loop on the injection valve. The Models 725 and 728 Autosamplers were capable of making up to three injections per vial while the new Model 708 can be programmed to make up to four injections per vial. The number of useable injections per vial depends not only on the amount of sample in the vial and the volume of the sample loop, but transfer system volume as well. Table 1 lists the transfer system volumes of the three models of Positive Displacement autosamplers made by Alcott/Micromeritics. The transfer system volume is the sum of the volumes of the needle, transfer line, and plumbing connections. The Model 725 used a zero dead volume union to connect the needle to the transfer line, while the Model 728 used the Needle Drive Block for this connection. The Model 708 uses a Valco, low dead volume bulkhead fitting.

The knowledge of these volumes is important in order to reduce carry-over and to insure good injection reproducibility. The volume of sample required to completely fill a length of tubing is approximately four times the tube's volume. This "4 x" factor is required to insure that the previous tube contents are completely rinsed from the tube. If there exists any chemical interaction between the liquid and the walls of the tubing, *i.e.* adsorption or if the previous solution was highly concentrated, additional rinsing will be necessary. The amount of sample required to make an injection with a Positive Displacement autosampler will be $4 \times$ (the transfer system volume + the loop volume). For the Model 708 with a 10 μL loop the required volume is $4 \times (48 + 10) = 232 \mu\text{L}$. If the Model 708 is programmed to make three injections per vial, 450 μL of the sample are transferred on the first injection, almost twice the required 232 μL . The second and third injections will require $4 \times$ the loop volume only, because the needle and transfer line already have sample in them. For the 10 μL loop, $4 \times 10 = 40 \mu\text{L}$ of sample are required to fill the loop and 140 μL are displaced each for both the second and third injections.

Table 2 lists the recommended numbers of injections per vial for given loop sizes. These recommendations are based on the calculations given above and have been verified experimentally. A copy of the experimental data is available from the Alcott Chromatography Applications Laboratory. These experimental recommendations are based on a vial being filled to within 6 mm (1/4 inch) from the top (the Alcott definition of a completely filled vial). Note that approximately 140 μL of sample is left in the vial under the cap after all the contents of the vial have been transferred to the valve! Using loop sizes above these recommended volumes will result in poor reproducibility and excessive carry-over! Also remember, these volumes may require modification if the sample has the potential for adsorbing onto the walls of the tubing.

Table 1: System Volumes (uL)

	Model 725	Model 728	Model 708
Needle	7.0	7.0	7.0
Transfer Line	66.5	30.9	40.5
Connector	0.0	13.0	0.5
Total	<hr/> 73.5	<hr/> 50.9	<hr/> 48.0

Table 2: Recommended Maximum Loop Volumes

Injections Per Vial	Maximum Loop Volume (uL)
1	200
2	100
3	75
4*	10

* Model 708 only