

***The Smallest, In-Vial Sample Volume Useable with  
Alcott Positive Displacement Autosamplers***

One of the most frequently asked questions about the Alcott/Micromeritics "Positive Displacement" type autosamplers concerns the minimum volume of sample required to make an injection. The answer to this question depends mainly on the volume of the sample loop used. Experience with manual, filled or "fixed" loop injectors has show that four to five loop volumes of sample are required to completely rinse the sample loop of its previous contents in order to ensure good injection precision. When using a Positive Displacement autosampler, the transfer system volume, in addition to the sample loop volume, must be taken into consideration as well. The Alcott Model 708 Autosampler has a transfer system volume of 48  $\mu\text{L}$ . This 48  $\mu\text{L}$  is the sum of the needle volume, transfer line volume, and the plumbing connection volume. Finally, one must also consider the volume of the sample left behind under the vial cap. This volume is approximately 140  $\mu\text{L}$ . To estimate the minimum amount of sample the following formula is used:

Minimum Sample Volume =

$$\{(The\ Transfer\ System\ Volume\ +\ The\ Loop\ Volume)\ \times\ 4\} + The\ Volume\ Under\ The\ Cap$$

Therefore, the estimated total amount of sample required to make a 10  $\mu\text{L}$  injection is  $\{(48 + 10) \times 4\} + 140$  which equals 372  $\mu\text{L}$  of sample. The word "estimated" is used because one must consider the air in the top of the vial which is initially displaced before the sample enters the transfer line and then the loop. This "slug of air" helps eliminate the previous sample and thereby improves loop rinsing and filling. The following experiments were conducted to illustrate the minimum sample required for a Model 708 Autosampler equipped with both a 10  $\mu\text{L}$  and a 20  $\mu\text{L}$  loop.

The conditions used for this experiment are those specified in the standard Alcott Chromatography Test Conditions described in the Model 708 User's Manual. The test sample was modified to contained both Methyl and Propyl Paraben. Forty vials of this test solution were prepared for the experiment. The vials were filled in groups of four to contain 900, 50, 100, 150, 200, 250, 300, 350, 400, and 900  $\mu\text{L}$  of test sample each. The Autosampler was programmed to make one injection per vial. A total of forty injections were made. The procedure was conducted with a Model 708 equipped with a 10  $\mu\text{L}$  loop and then repeated with the same instrument equipped with a 20  $\mu\text{L}$  loop. The table on the reversed side shows the experimental data listed in Peak Area measurements for both the Methyl and Propyl derivatives. Also listed are the average Peak Areas for each volume as well as the Standard Deviation and Relative Standard Deviation (RSD) for each volume.

It can be seen from looking at the data for both loop sizes, that nothing is injected when a sample volume of 150  $\mu\text{L}$  or less is placed in the vial. This would be expected since the volume under the vial cap is 140  $\mu\text{L}$ . At 200  $\mu\text{L}$  of solution in the vial, sample is injected, but only about half of the loop is filled, and not very consistently, as seen by the large RSD value. At 300  $\mu\text{L}$ , the Peak Area starts to reach the expected value for the loop size, but the RSD is still above the specified 0.30%. Finally at sample volumes of 350  $\mu\text{L}$  or greater, the RSD values start to fall below 0.30%. Note that even though the calculated minimum volume of sample for the 10  $\mu\text{L}$  loop was 372  $\mu\text{L}$ , 350  $\mu\text{L}$  of sample gave good RSDs. This is the result of the approximately 600  $\mu\text{L}$  "slug of air" passing through the needle, transfer line, and loop first! The 20  $\mu\text{L}$  loop required  $\{(48 + 20) \times 4\} + 140 = 412$   $\mu\text{L}$  of sample, but again, 350  $\mu\text{L}$  of sample coupled with the approximately 600  $\mu\text{L}$  of air was sufficient to provide good results.

In closing, note that these results were obtained using an "ideal" test sample. Highly concentrated samples, which require may more than 4 or 5 loop volumes to thoroughly rinse the loop, or samples which interact with any of the wetted surfaces within the Autosampler, may require larger volumes to adequately wash the loop in order to improve reproducibility or to reduce carry-over.

*Injection Precision and Total Sample in Vial Volume 10 uL Loop*

**Volume of Sample**

*in the Vial --->*

	900 uL		50 uL		100 uL		150 uL		200 uL	
	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>
	1234381.00	1035750.00	0.00	0.00	0.00	0.00	0.00	0.00	481315.00	394129.00
	1227067.00	1030705.00	0.00	0.00	0.00	0.00	0.00	0.00	249088.00	221339.00
	1231720.00	1033788.00	0.00	0.00	0.00	0.00	0.00	0.00	407927.00	358563.00
	1235102.00	1036512.00	0.00	0.00	0.00	0.00	0.00	0.00	1060514.00	971184.00
<b>Average</b>	1232067.50	1034188.75	0.00	0.00	0.00	0.00	0.00	0.00	549711.00	486303.75
<b>Std. Dev.</b>	3637.13	2590.50	0.00	0.00	0.00	0.00	0.00	0.00	354059.60	331727.33
<b>RSD (%)</b>	0.30	0.25	0.00	0.00	0.00	0.00	0.00	0.00	64.41	68.21

**Volume of Sample**

*in the Vial --->*

	250 uL		300 uL		350 uL		400 uL		900 uL	
	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>
	1212363.00	1031598.00	1232116.00	1040693.00	1244683.00	1044642.00	1248286.00	1047818.00	1234848.00	1036893.00
	1231898.00	1029518.00	1238541.00	1034047.00	1245558.00	1047581.00	1246572.00	1049337.00	1236914.00	1037605.00
	1224274.00	1004146.00	1247152.00	1045772.00	1243877.00	1042726.00	1244115.00	1045005.00	1234823.00	1039680.00
	1224287.00	1022196.00	1228219.00	1040443.00	1242458.00	1043594.00	1243180.00	1045166.00	1235872.00	1037274.00
<b>Average</b>	1223205.50	1021864.50	1236507.00	1040238.75	1244144.00	1044635.75	1245538.25	1046831.50	1235614.25	1037863.00
<b>Std. Dev.</b>	8071.16	12481.62	8274.96	4802.87	1317.04	2114.00	2324.20	2110.35	994.82	1245.78
<b>RSD (%)</b>	0.66	1.22	0.67	0.46	0.11	0.20	0.19	0.20	0.08	0.12

*Injection Precision and Total Sample in Vial Volume 20 uL Loop*

**Volume of Sample**

*in the Vial --->*

	900 uL		50 uL		100 uL		150 uL		200 uL	
	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>
	2384188.00	2009023.00	0.00	0.00	0.00	0.00	0.00	0.00	399091.00	318368.00
	2386932.00	2006613.00	0.00	0.00	0.00	0.00	0.00	0.00	319467.00	256655.00
	2385245.00	2005167.00	0.00	0.00	0.00	0.00	0.00	0.00	318134.00	253174.00
	2395480.00	2007520.00	0.00	0.00	0.00	0.00	0.00	0.00	988241.00	810866.00
<b>Average</b>	2387961.25	2007080.75	0.00	0.00	0.00	0.00	0.00	0.00	506233.25	409765.75
<b>Std. Dev.</b>	5138.30	1617.25	0.00	0.00	0.00	0.00	0.00	0.00	323560.35	269071.76
<b>RSD (%)</b>	0.22	0.08	0.00	0.00	0.00	0.00	0.00	0.00	63.92	65.66

**Volume of Sample**

*in the Vial --->*

	250 uL		300 uL		350 uL		400 uL		900 uL	
	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>	<i>Methyl</i>	<i>Propyl</i>
	2404146.00	2010893.00	2412645.00	2025210.00	2410011.00	2018356.00	2413879.00	2017623.00	2398765.00	2008105.00
	2405856.00	2008708.00	2406531.00	2027608.00	2422373.00	2025091.00	2407753.00	2018150.00	2395217.00	2007920.00
	2380944.00	1992778.00	2396583.00	2006637.00	2407261.00	2021551.00	2419931.00	2028098.00	2396998.00	2003024.00
	2406740.00	2015861.00	2416325.00	2019788.00	2418282.00	2027232.00	2415120.00	2023439.00	2396316.00	2002035.00
<b>Average</b>	2399421.50	2007060.00	2408021.00	2019810.75	2414481.75	2023057.50	2414170.75	2021827.50	2396824.00	2005271.00
<b>Std. Dev.</b>	12365.30	9980.66	8629.13	9371.89	7043.69	3913.02	5011.79	4936.87	1487.53	3192.15
<b>RSD (%)</b>	0.52	0.50	0.36	0.46	0.29	0.19	0.21	0.24	0.06	0.16

