

## Liquid Chromatography

# Use of Blunt-Tip Needle on Flexar-Series Autosamplers for Very-Low Volume Samples

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## Introduction

In quite a number of labs, it is important that one be able to inject low  $\mu\text{L}$  volumes out of already low  $\mu\text{L}$  samples. This is especially common in biotech labs, where a purified sample to be analyzed may total no more than 50  $\mu\text{L}$ . Not only cannot such samples be wasted, quantitatively, they must be sampled reliably. To afford this, it is important that the needle inlet is at the very tip, instead of slightly up the side. The example below highlights the achievable results when using such a “blunt- tip” needle on a PerkinElmer Flexar LC system.

## Experimental Conditions

Table 1. HPLC conditions/configurations.

<b>System</b>	Chromera/Flexar FX UHPLC UV/Vis System
<b>Column</b>	Brownlee Pecosphere CRC18 3.3 x 3 cm, 3 $\mu\text{m}$
<b>Solvent</b>	18% ACN/Water, at 1 mL/min
<b>Sample</b>	150 ppm Caffeine
<b>Vial Type</b>	2 mL Vial with 150 $\mu\text{L}$ Insert; 3 mm Needle Height
<b>Injection Mode</b>	$\mu\text{L}$ -Pickup Mode Using Slow Sample Speed with 20 $\mu\text{L}$ loop; 250 $\mu\text{L}$ Post-inj. Wash
<b>Injection Volume</b>	3 and 5 $\mu\text{L}$ out of 6 $\mu\text{L}$
<b>Blunt-tip Needle</b>	Part#: N2931199 (Optional For All Flexar-series/225/275 Autosamplers)

## Results

As shown in Table 2, below, using the blunt-tip needle, when injecting 3  $\mu\text{L}$  out of 6  $\mu\text{L}$ , the area reproducibility for six replicates of 150  $\mu\text{L}$  caffeine standard is just over 0.8% RSD.

Table 2. RSD of six caffeine std. replicates, injecting 3  $\mu\text{L}$  out of 6  $\mu\text{L}$ , using blunt-tip needle.

Sample Name	Caffeine
	Area
Caff-uLPick-BluntNeedle001	1,365,719.3
Caff-uLPick-BluntNeedle002	1,348,025.1
Caff-uLPick-BluntNeedle003	1,371,864.3
Caff-uLPick-BluntNeedle004	1,378,699.0
Caff-uLPick-BluntNeedle005	1,376,177.9
Caff-uLPick-BluntNeedle006	1,375,937.8
Average	1,369,403.9
% RSD	0.834

Even more demanding, as shown in Table 3, below, when injecting 5  $\mu\text{L}$  out of 6  $\mu\text{L}$ , the area reproducibility for six replicates of this caffeine standard is just over 1.3% RSD.

Table 3. RSD of six caffeine std. replicates, injecting 5  $\mu\text{L}$  out of 6  $\mu\text{L}$ , using blunt-tip needle.

Sample Name	Caffeine
	Area
Caff-uLPick-BluntNeedle001	2,273,495.3
Caff-uLPick-BluntNeedle002	2,268,617.6
Caff-uLPick-BluntNeedle003	2,222,129.5
Caff-uLPick-BluntNeedle004	2,268,397.5
Caff-uLPick-BluntNeedle005	2,279,547.3
Caff-uLPick-BluntNeedle006	2,318,649.3
Average	2,271,806.1
% RSD	1.356

Finally, the results in Table 4 show exceptional RSD performance (0.20%) when using the blunt-tip needle for 3  $\mu\text{L}$  injections out of 300  $\mu\text{L}$ . Though not shown here, both area responses and RSD values compared quite closely with those obtained using the standard needle (0.32% RSD).

Table 4. RSD of six caffeine std. replicates, injecting 3  $\mu\text{L}$  out of 300  $\mu\text{L}$ , using blunt-tip needle.

Sample Name	Caffeine
	Area
Caff-uLPick-BluntNeedle001	1,846,280.4
Caff-uLPick-BluntNeedle002	1,843,854.5
Caff-uLPick-BluntNeedle003	1,852,320.5
Caff-uLPick-BluntNeedle004	1,842,390.5
Caff-uLPick-BluntNeedle005	1,843,131.7
Caff-uLPick-BluntNeedle006	1,847,558.6
Average	1,845,922.7
% RSD	0.200

## Conclusion

Based on the results, though it is always best, whenever possible, to inject small volumes out of larger sample volumes, if one is sample-limited, exceptionally good and reproducible results can be obtained by injecting out of very low sample volumes. This is afforded by using a blunt-tip needle option, in which the lower needle orifice is situated at the very tip of the needle. For 3  $\mu\text{L}$  injections out of 6  $\mu\text{L}$ , RSDs of less than 1% are achievable and for 5  $\mu\text{L}$  injections out of 6  $\mu\text{L}$ , RSDs of less than 2% are quite achievable. Using the blunt-tip needle with micro-vial inserts, the minimum sample limit is 6  $\mu\text{L}$ , compared to ~15  $\mu\text{L}$ , using the standard needle.

Also, performance-wise, the blunt-tip needle compares quite favorably to the standard needle, in both area response and RSD.