Accurate Measure of Injection Volumes with Scion 8400 AutoSampler

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Computation of analytical results in gas chromatography usually involves direct comparisons of area counts for target compounds in the unknown sample with corresponding areas for standards, run under identical conditions. Since the two areas are ratioed, accurate aliquots of the injection volumes are not critical, as long as they match. If, for example, the target injection is 1.0 microliters, for both standard and unknown, but instead the injected volume is slightly different, say 1.3 microliters, the change in volume is canceled out in the calculation. Instead, critically important in the experiment is the reproducibility of the injection volume. Any discrepancy in volumes will directly the quality of the results. Automated processes for injecting samples into the gas chromatograph greatly improves the precision of the injection volume.

However, circumstances can occur where the accuracy of the specified injection volume plays an important role in the experiment. Examples include:

- Direct comparisons of results with and without internal standard addition.
- Target concentration is bracketed with different injection volumes of the same standard.
- Measurement of extraction efficiencies of purge-trap or solvent extraction with conventional injection of matching liquid standards.
- Multi-point calibration by injecting different volumes of the same standard.

This error occurs because the syringe can have the specified volume in the syringe barrel plus residual sample remaining in the syringe needle just before injection, as illustrated in Figure 1. Typical needles attached to 10 microliter syringes contain 0.7 microliters of sample. When the syringe contents are injected, the actual volume dispensed into the hot injector becomes the desired dosage plus additional amount due to boiling off from residual sample contained in the needle. This amount is very dependent on how long the syringe remains in the injector, the boiling point of the solvent used, and the temperature of the injector. Typically, this residue lost amounts to 0.4 microliters.

To rectify this problem, the Scion 8400 AutoSampler can be preprogrammed to initially insert an air gap into the syringe, a dose of solvent, another air gap, then the sample loading, and finally a third air gap. Figure 3 shows the steps involved. This sequence provides assurances that the full aliquot of the sample is injected, and air gaps and solvent dose flush out the syringe.

After the autosampler sequence is set up to perform pre-injection solvent clean flushes, the syringe needle ends up full of residue solvent, as depicted in Figure 3. This action does not pose any change in the sample dose, as with steps discussed in Figure 1.

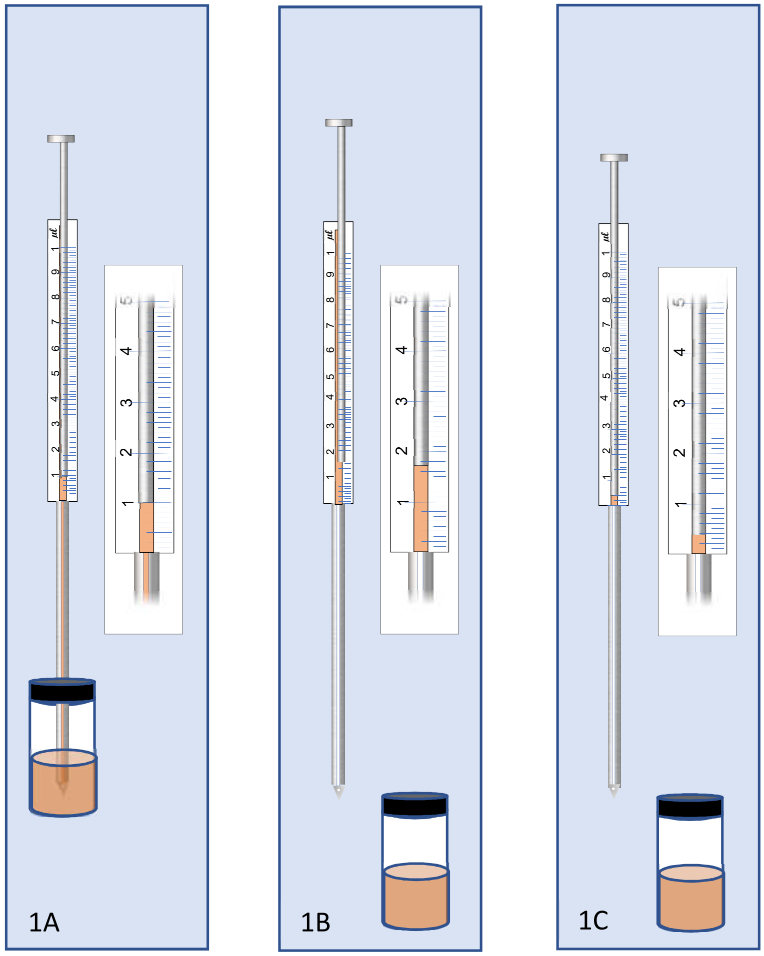

Figure 1. Typical sequence of sample loading and injection is shown in this pictorial series. In 1A, the syringe barrel and needle are fully flushed and then the aliquot is set to the desired volume of 1.0 microliter. If the plunger is now pulled back (in 1B), the total volume is shown as 1.7 microliters. After injection, the residue sample is 0.4 microliters, with extra sample pulled out from the heat of the injector. The effective volume injected is 1.3 microliters, or a +30% error from the requested volume.

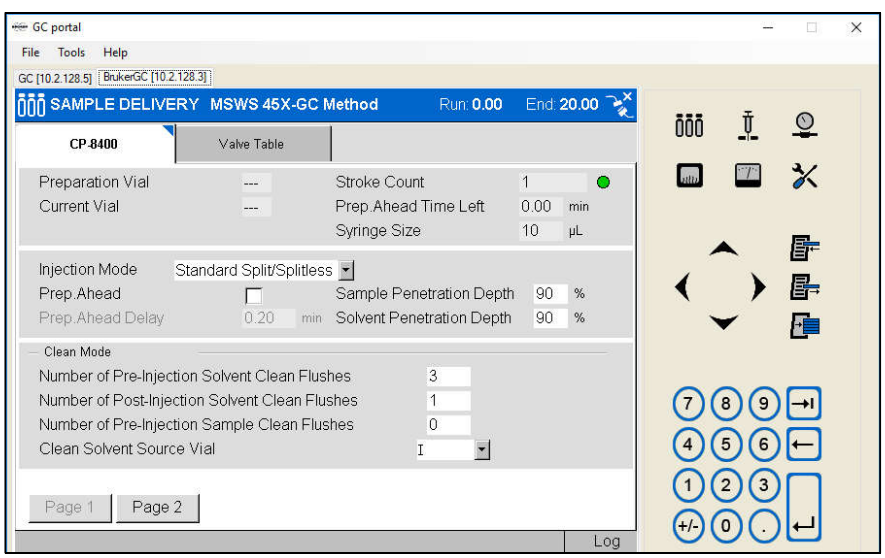

Figure 2. AutoSampler parameters for operations in Figure 1.

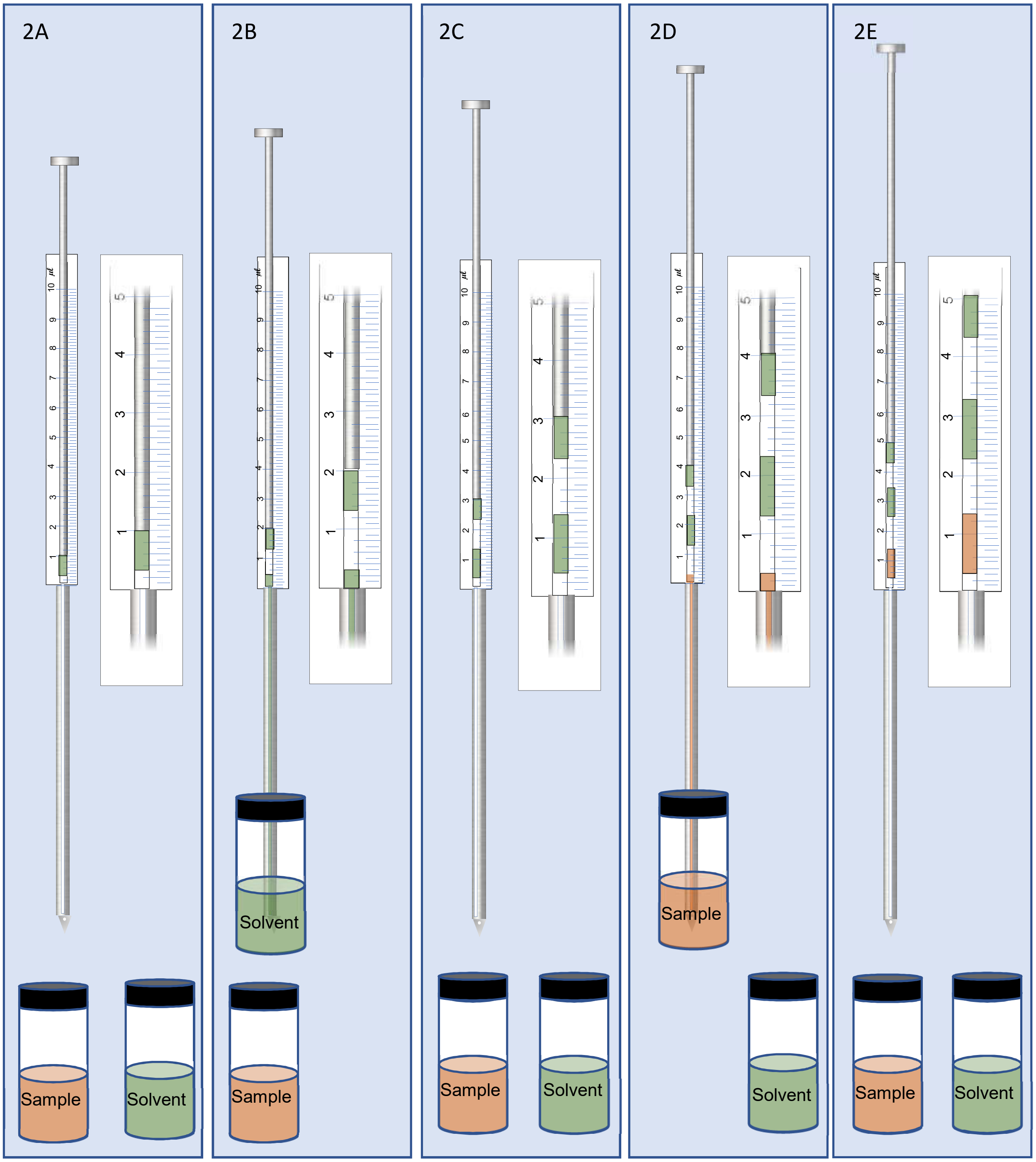

Figure 3. Setting up User Defined parameters for the Scion 8400 AutoSampler gives a sample loading sequence depicted, with insertions of air gaps and solvent flush to provide a full loading of the specified volume. In 2A, the first step is to dose in 1.0 microliters of room air, which becomes a full 1.0 microliter including the volume in the needle. Step 2B then loads up 1.0 microliter of solvent. Step 2C pulls in 1.0 microliter of air to provide a barrier between the solvent and the sample. Step 2D extracts the specified sample volume from the vial. Finally, a third air gap (2E) ensures that no sample persists in the syringe after injection.

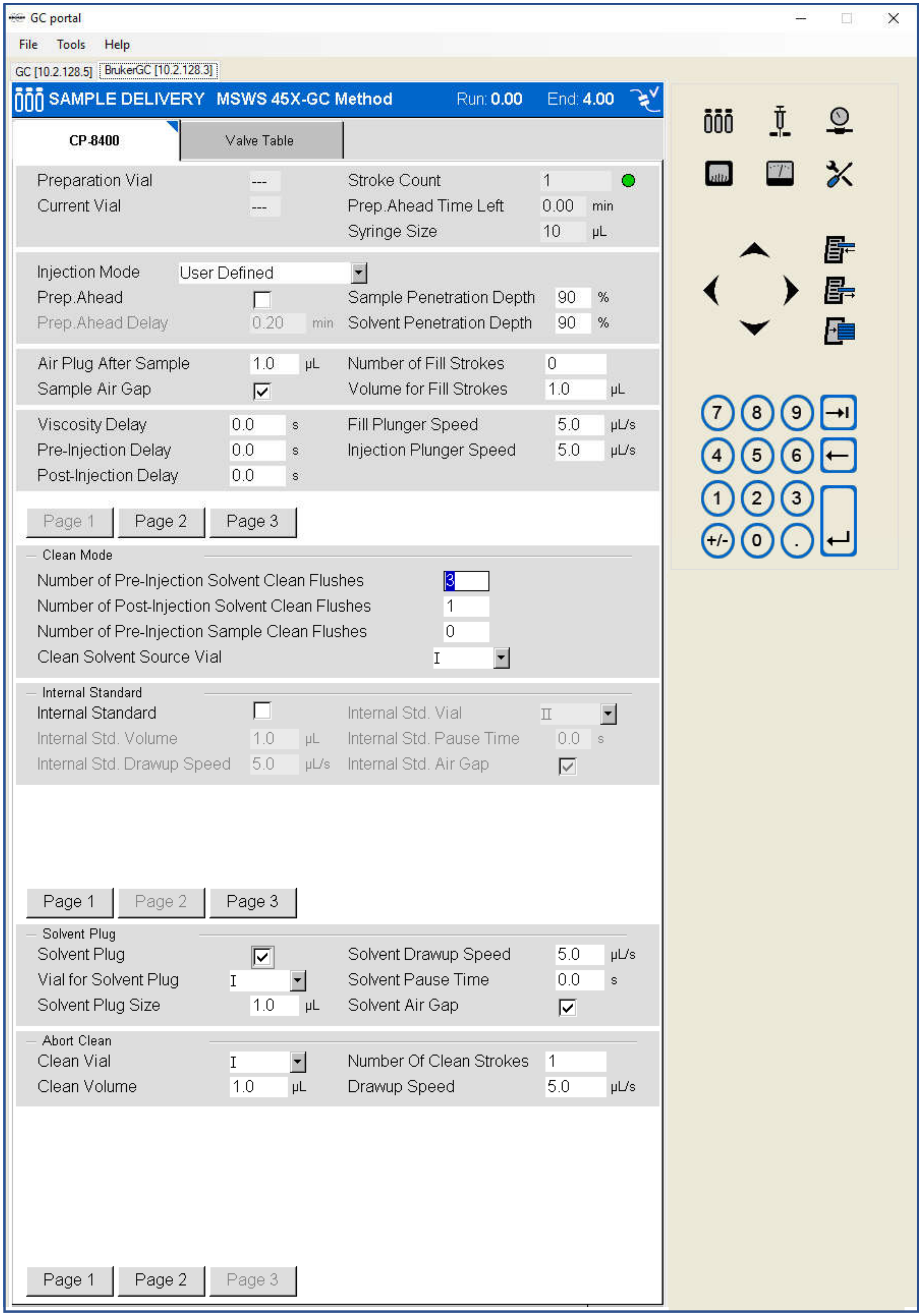

Figure 4. AutoSampler parameters for operations in Figure 3.

The errors realized when using the standard Split/Splitless injection mode is demonstrated in Figure 5. The peak size for lindane is 30 percent larger than when a precise 1.0 microliter is loaded with User Defined parameters listed in Figure 4. The error increases when smaller volumes are specified, as the "hidden" volume in the needle becomes a larger contribution to the effective injection volume.

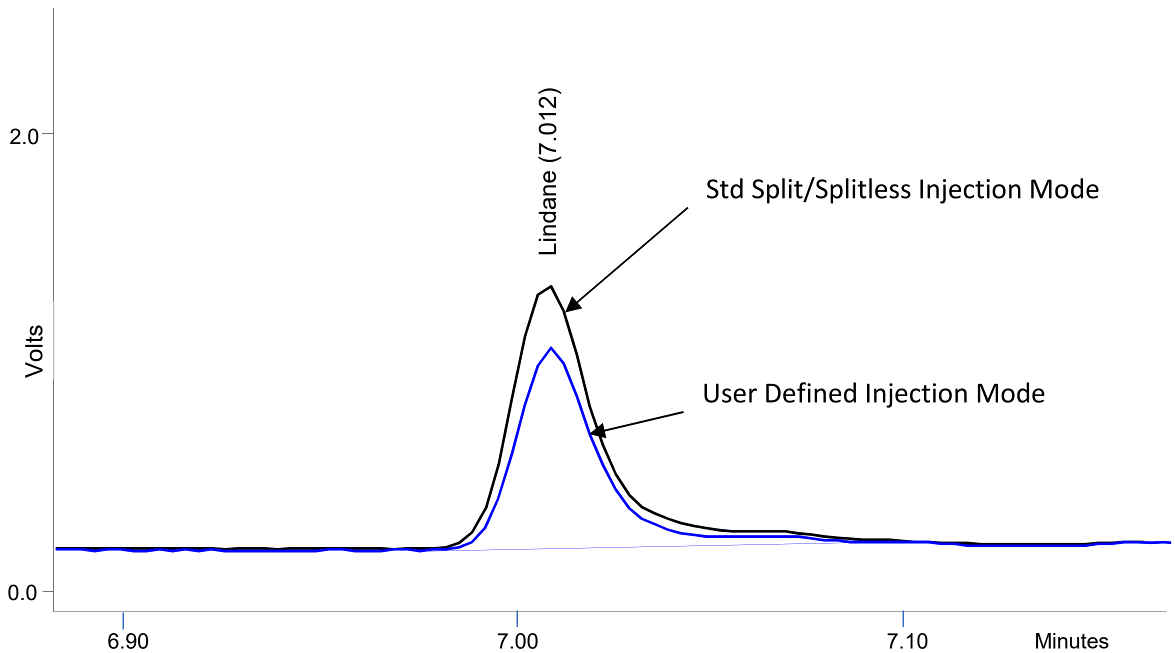

Figure 5. Comparison of 33 ug/ul lindane chromatograms with Std Split/Splitless Injection Mode versus User Defined Injection Mode applying parameters listed in Figure 4. Targeted injection volume is 1.0 microliter.

A useful capability of properly loading up different injection volumes is the generation of a calibration curve from a single standard. Simply by listing different injection volumes in the sample list from a single vial, achievable results are shown in Figure 6 for lindane. Injection volumes were varied from 0.1 to 0.5 microliters with a 10-microliter syringe.

A similar operation can be performed to bracket sample target concentration with two standards just above and just below the expected concentration simply by stipulating different injection volumes.

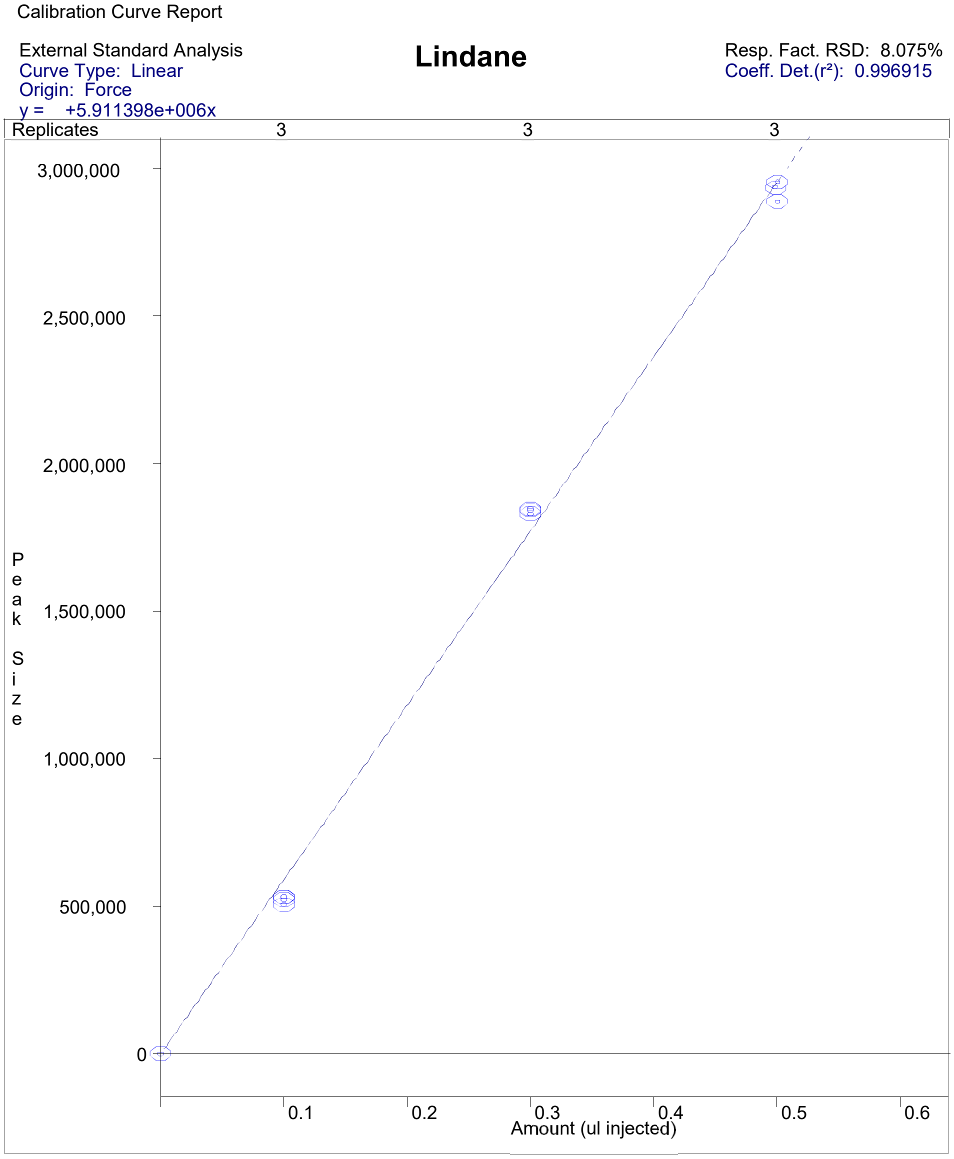

Figure 6. Calibration curve for 33 µg/µL lindane in iso-octane, generated by using various injection volumes of a single standard with AutoSampler parameters listed in Figure 4.