Technical Report

Investigation on Improving the Reproducibility of Nanoliter Injections Using a Standard Syringe
Getting more out of your standard consumables

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Abstract:

Nanoliter liquid injections are usually carried out using a plunger-in-needle syringe, a syringe wherein the plunger traverses the syringe body all the way to the tip of the needle. However, these syringes can be expensive, difficult to handle and require frquent maintenance. In this report, the feasibility of using a standard syringe to inject nanoliter amounts of sample was investigated. Initial results show that standard syringes suffer from poor repeatability. However, it was found that repeatability can be significantly improved by extending the post injection dwell time.

Keywords: AOC-30i, nanoliter injection, syringe

1. Introduction

Advances in GC sampling and detection technologies, have enabled scientists to measure smaller and smaller quantities of sample. Liquid samples are typically introduced using a microliter syringe, which can deliver several microliters to nanoliters of sample into the sample inlet.

For many applications, which do not require trace level analysis, introducing smaller amounts of sample can be more efficient and more economic. Smaller injection volumes lead to smaller vapor volumes. This reduces the risk of sample overflowing and exceeding the liner's effective volume, which can lead to difficult problems like contamination, double injection and others. Decreasing the amount of sample in contact with the liner, can also help extend the liner's life. Longer life translates to less frequent maintenance, which directly translates to more injections.

This sounds simple in principle, but it is not without its problems. One major problem with injecting very small sample volumes is achieving good repeatabilities. Typical microliter syringe needles have several hundred nanoliters of dead volume, which makes it difficult to dispense minute volumes of sample accurately. This problem can be remedied by using a special type of syringe with a finely fitted wire, which traverses the syringe body all the way to the tip of the needle, commonly known as a plunger-in-needle syringe. This syringe has almost zero dead volume but can be quite expensive and difficult to maintain. Another limitation is that most designs only allow the injection of very narrow range of volumes (e.g., 0.05 µL to 0.5 µL).
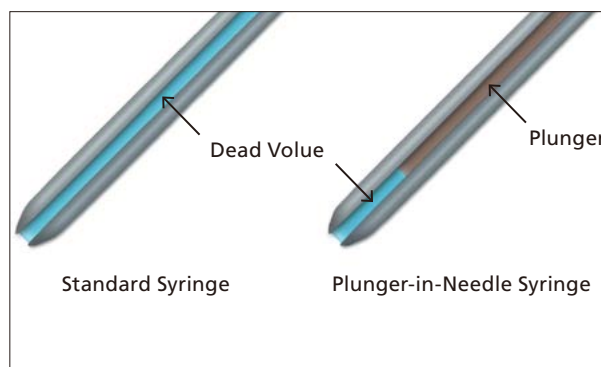

Fig. 1   Syringe needle of a standard syringe vs a plunger-in-needle syringe

Using a plunger-in-needle syringe can help achieve nanoliter injections with good repeatability. However, these can be expensive and difficult to maintain. In this report, we illustrate how extending the post injection dwell time can be used to improve peak area repeatabilities of nanoliter injections using standard syringes.

2. Experiment

A sample consisting of 100 ppm (v/v) n-dodecane (C12), n-tetradecane (C14) and n-hexadecane (C16) in hexane was injected 10 consecutive times into a sample inlet. Each experiment consisted of using a different type of syringe and varying the injector settings. The relative standard deviation of the peak areas were calculated after each set. In another experiment, a mixture containing 100 ppm octanol and Dicyclohexylamine in hexane (v/v) was evaluated using similar conditions as the first experiment.

In order to gain insight into the consistency of the injections, data were summarized into a series of box plots. A box plot allows one to graphically evaluate the dispersion of the data, which is a measure of the repeatability of the measurements. The height of the box correlates with the amount of dispersion in the data. More precise measurements yield narrower boxes. A simple explanation of a typical box plot is shown in Fig. 2. In principle, consistent data should yield narrow boxes that are symmetric in reference to the median.
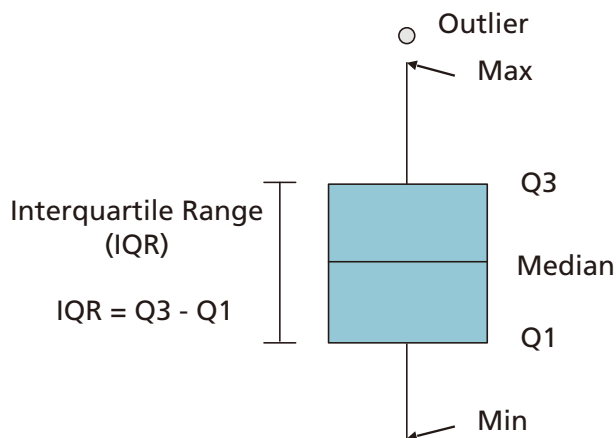

Fig. 2   Example box plot with labels

The following are the setup and method parameters used in the experiment.

Table 1   Method Parameters

Model	: Nexis™ GC-2030/AOC-30i
Injection Volume	: 0.1 µL~1 µL
Injection Temp.	: 250 °C
Injection Mode	: Split
Split Ratio	: 39.5
Carrier Gas	: He
Flow Control Mode	: Linear Velocity (87.1 cm/s)
Column	: SH-I-1HT Cap. (PN: 227-36089-01)
	(15 m x 0.32 mm I.D., 0.10 µm)
Column Temp.	: 120 °C (3 min)
Detector	: FID-2030
Detector Temp	: 300 °C
Detector Gas	: H2 32.0 mL/min, Air 200 mL/min
Makeup Gas	: N2 24.0 mL/min

Injector Settings	
Pre Wash	: A x 1
Post Wash	: A x 2
Sample Wash	: 2
Plunger Speed	: Fast
Inj. Port Dwell Time	: 0 (default)

Syringes Used	
PN: 227-35002-01	0.5 µL syringe
PN: 221-75173	5 µL syringe
PN: 221-34618	10 µL syringe

3. Results

A 0.5 µL plunger-in-needle syringe, a 5 µL and a 10 µL standard syringe were used to take 10 consecutive injections of a standard sample. For each experiment, the peak area repeatability was calculated, and the results were plotted in a box plot to evaluate the consistency of the measurements. Furthermore, a subsequent experiment using a 5 µL standard syringe, where the post injection dwell time was varied from 0 to 5 seconds was performed to test whether improvements in peak area repeatability could be achieved.

3.1 Repeatability comparison based on syringe type

The boxplot below shows the distribution of peak area repeatabilities (RSD) for n-dodecane when using different types of syringes (0.5 µL plunger-in-needle syringe, 5 µL syringe, 10 µL syringe) to inject 0.1 µL of sample. The 4th box shows data that uses a standard 5 µL syringe but utilizes a method that extends the post injection dwell time by 5 seconds. Each point in the plot is the RSD value for 10 consecutive injections.
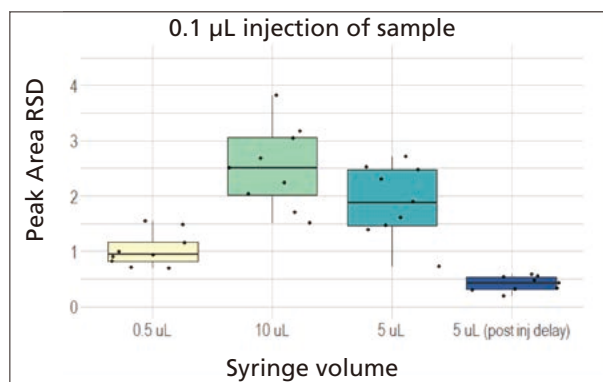

Fig. 3   Boxplot showing the distribution of RSD values for a 0.1 µL injection of sample obtained using different syringes; data shown is for n-dodecane. Each point on the plot is the RSD value for 10 injections. The total number of data sets is 9. Some randomness "jitter" was added to the points along the x-axis to make the points more visible.

The data in Fig 2 shows that in general, using a syringe with smaller capacity can help improve peak area repeatabilities when injecting small amounts of sample. Furthermore, using a plunger in needle syringe (0.5 µL) with near zero dead volume, can yield peak area repeatabilities of around 1%. The experiment also shows using a standard syringe and extending the post injection dwell time, results in a drastic improvement in peak area repeatability. This improvement can be attributed to more efficient volatilization of sample in the syringe needle due to the extended time the syringe needle is exposed to the heated inlet. Although this method can improve peak repeatabilities it also has its drawbacks, which will be discussed in the following sections.

3.2 Things to consider when using a 0.5 µL plunger-in-needle syringe

The results in the previous section illustrate that improvements in peak area repeatability can be achieved by using a plunger-in-needle syringe. For applications that require high precision and fast injection of nanoliter amounts of sample, the plunger-in-needle syringe can be used to obtain good results.

It is appropriate for samples that are thermally labile and need to be exposed to the heated inlet in the shortest amount of time possible. In addition, it is also suitable for samples that have a wide range of boiling points, as these types of samples are prone to sample discrimination. The plunger-in-needle syringe certainly has its place. However, due to the nature of this syringe it also has several limitations.

Due to the syringe's construction, it is more costly and more complicated to operate than a standard syringe. Furthermore, because of its size, the syringe can only accommodate a narrow range of sample volumes. In cases, where the specific qualities of the plunger-in-needle syringe are not required and supply and price constraints are a factor, a standard syringe can be used although with some caveats.

3.3 Effectiveness of extended post injection delay on the injection of nL amounts of different compounds.

As stated in the previous section, the proposed method may not be ideal for all compounds, in this section we explored its effectiveness when dealing with some commonly used compounds.

3.3.1 Case for n-alkanes with similar boiling points

0.1 µL of a sample containing 100 ppm of n-dodecane (C12), n-tetradecane (C14) and n-hexadecane (C16) in hexane was injected consecutively into a heated inlet and analyzed with the GC method listed in Table 1; the post injection delay time was set to 5 seconds.

A total of 15 sets (1 set consists of 10 injections) were analyzed and the repeatability (RSD) for each set was calculated. The results can be found in Fig 4.

The results reveal that for the analysis of common alkanes, increasing the post injection delay time significantly improves the peak area repeatability. The median values for each compound is less than 0.5% RSD.
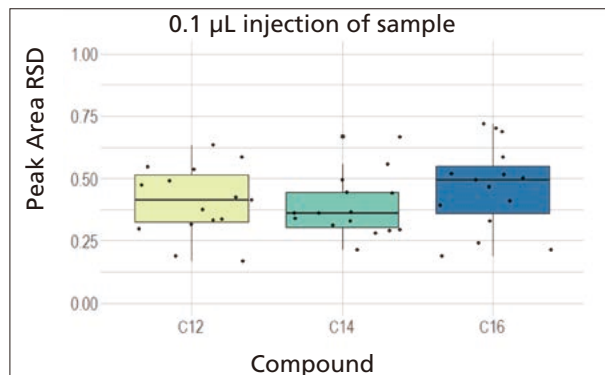

Fig. 4   Boxplot showing the distribution of RSD values for n-dodecane, n-tetradecane and n-hexadecane obtained using a 5 µL syringe and setting the post injection dwell time to 5 s. Each point on the plot is the RSD value for 10 injections. The total number of data sets is 15. Some randomness "jitter" was added to the points along the x-axis to make the points more visible.

3.3.2 Case for octanol and dicyclohexylamine

The effectiveness of the method was also tested on other compound classes. In this work, octanol (alcohol) and dicyclohexylamine (amine) were tested.

The results reveal that the method was also effective for these compounds. The calculated peak area repeatabilities were between 0.3% – 0.4% RSD for both compounds.

3.4 Influence of post injection dwell time on different injection volumes

It is proposed that by extending the post injection dwell time, sample present in the needle's dead volume will also be volatized, leading to more sample being introduced into the inlet. Fig 5. shows the response factors (peak area/injection volume) for different injection volumes (0.1 µL to 1 µL) when subject to a post injection dwell time of 3 seconds. The syringe used was a 5 µL microsyringe.
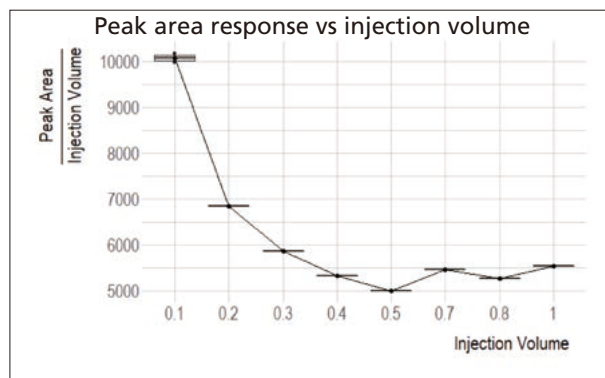

Fig. 5   Injection volume vs peak area/injection volume for n-dodecane. Post injection dwell time was set to 3 seconds.

As shown in Fig 5., the response factor is much larger for smaller injection volumes. This effect plateaus from 0.4 µL onwards. The increase in response factor for smaller injection volumes is due to the volatilization of sample present in the needle's dead volume. This leads to an increase in the amount of sample introduced into the inlet.

Because of this effect, it is important to exercise caution when using this technique, especially when comparing different injection volumes or analyzing peaks that are not well separated. On the other hand, analysis which are not sensitive to the above-mentioned changes in response factor, can take advantage of this technique to yield improved repeatabilities while reducing contamination by reducing the amount of sample introduced into the inlet.

4. Conclusion

Reducing the injection volume when feasible, can have various benefits, particularly on extending consumable lifetime and reducing frequency of routine maintenance. The most common method for injecting nanoliter amounts of liquid sample is to use a plunger-in-needle syringe. However, these can be costly and require high maintenance. An alternative presented in this report is to use a standard syringe but extend the duration the needle is exposed to the sample inlet. The technique results in a significant improvement in peak area repeatability for nanoliter injections of various compounds. However, due to the nature of the technique, not all compounds should be used, especially thermally labile and sensitive compounds. Although repeatability improves, the peak area response can also vary depending on the injection volume, therefore it is advised that the technique be used on a case-by-case basis. It is recommended that a screening experiment be carried out on a test sample before using the technique for analysis.

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