

# Improved Data Quality Through Automated Sample Preparation

## Application Note

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### Abstract

Sample preparation tasks can be extremely time-consuming and are often prone to errors, leading to poor reproducibility and accuracy. Many of these tasks, such as calibration curve generation, sample dilution, internal standard addition, or sample derivatization are performed daily, requiring significant resources as well. The Agilent 7696 Sample Prep WorkBench can perform many common sample prep tasks with better accuracy and precision than most manual methods, while using significantly fewer reagents and requiring less time from the operator. To demonstrate this, three sample preparation tasks were adapted for use on the Agilent 7696 Sample Prep WorkBench and yielded the same, if not better, results than the manual methods for accuracy and precision.



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## Introduction

The Agilent 7696 Sample Prep WorkBench can perform many sample preparation tasks for either gas chromatographic (GC) or liquid chromatographic (LC) analyses. The Agilent 7696 Sample Prep WorkBench consists of two liquid dispensing modules, a single vial heater capable of reaching 80 °C, a single vial mixer, and barcode reader (Figure 1). This enables dilutions/aliquoting, liquid addition, heating for derivatization or digestion, liquid/liquid extractions, and sample mixing. Individual racks can also be heated and/or cooled. This sample preparation instrument can perform tasks with the same accuracy and precision as the Agilent 7693A Automatic Liquid Sampler only in an offline setting instead of on top of a GC [1]. Many sample preparation tasks such as sample dilution, calibration curve standard generation, and sample derivatization within both fields can be time consuming and resource intensive. Automating these procedures with the Agilent 7696 Sample Prep WorkBench therefore is beneficial in many ways.



Figure 1. The Agilent 7696 Sample Prep WorkBench.

A side-by-side comparison of manual and automated methods was performed for three common sample prep applications to demonstrate the improved data quality achieved through automated sample preparation. Sample dilution, calibration curve standard generation, and derivatizations were performed with success on the Agilent 7696 Sample Prep WorkBench.

## Experimental

Three common sample preparation tasks were performed with the Agilent 7696 Sample Prep WorkBench. First, sample dilutions and internal standard additions were performed for analysis by both GC and LC. For the GC samples, 50  $\mu\text{L}$  each of isooctane and a standard solution containing four analytes were added to an empty 2-mL autosampler vial. Additionally 0.5  $\mu\text{L}$  of an internal standard solution (ISTD) containing three analytes was added to the vial. The solution was mixed using the onboard mixer before transferring the vials to a GC for

analysis. The samples for LC followed a similar procedure. To an empty 2-mL autosampler vial, 187.5  $\mu\text{L}$  of acetonitrile, 62.5  $\mu\text{L}$  of a pesticide standard, and 125  $\mu\text{L}$  of an ISTD were added. The sample was mixed before being transferred to an LC for analysis. For both of these sample dilutions,  $n=10$ .



Figure 2. The Agilent 7696 Sample Prep WorkBench with a gas chromatograph and mass spectrometer.

Second, generic calibration curves for the GC were made in triplicate via linear dilution both manually in 10-mL volumetric flasks and with the Agilent 7696 Sample Prep WorkBench. To make the standards manually, small amounts of hexane was added to six clean, dry 10-mL volumetric flasks. Varying amounts of a stock solution containing five analytes at 5 mg/mL, ranging from 0.1 to 1 mL, were added using serological pipets. The flasks were diluted to the mark with hexane to yield concentrations of 50, 100, 200, 300, 400, and 500 ppm. For the automated method, 100  $\mu\text{L}$  of hexane was added to six empty 2-mL autosampler vials. Again, varying amounts of the stock solution, ranging from 1 to 10  $\mu\text{L}$ , was added to the vials yielding approximately the same concentrations.

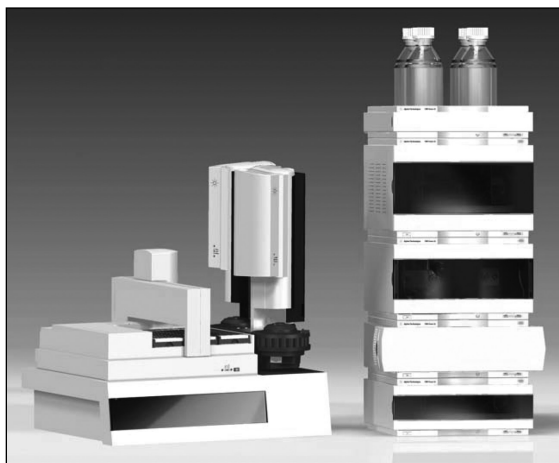


Figure 3. The Agilent 7696 Sample Prep WorkBench with a liquid chromatograph.

Third, derivatization of fatty acids via silylation reaction was performed. For the manual prep, 100  $\mu\text{L}$  of a silylating reagent was added to approximately 0.5 mL of a free fatty acid solution using an automatic pipettor. The solutions were heated to 70  $^{\circ}\text{C}$  using a heated block. The same derivatization was performed with the Agilent 7696 Sample Prep WorkBench using the single vial heater.

## Results and Discussion

### GC and LC Sample Dilution

For the 10 samples diluted for GC and LC analysis, the dispensed solvent, standard solution, and ISTD, was measured

gravimetrically to determine the reproducibility of the dispensing action. Dispensing 50  $\mu\text{L}$  with a 250  $\mu\text{L}$  syringe results in a 0.5% relative standard deviation (RSD) for the 10 samples measured by weight. The samples were diluted within 1% accuracy, determined from the peak areas. The ISTD exhibited a slightly higher RSD. Dispensing 0.5  $\mu\text{L}$  with a 25  $\mu\text{L}$  syringe resulted in an RSD of 2% for the 10 samples. If a smaller syringe had been used to dispense the ISTD, a lower RSD, closer to that obtained when dispensing the solvent and standard, would have resulted. The added ISTD did not affect the accuracy of the diluted sample (Figure 4).

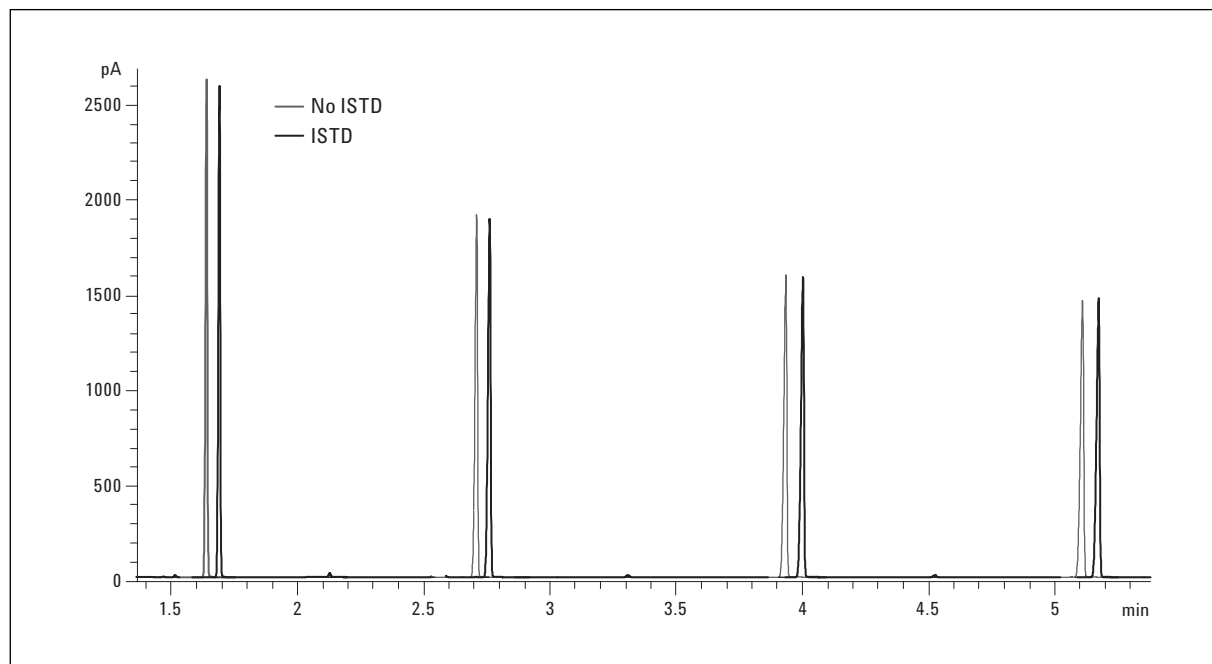


Figure 4. GC chromatograms (slightly offset) are shown for a standard solution dispensed and diluted with and without an ISTD added. No difference in peak areas are observed.

For the 10 samples diluted for LC analysis, similar results were obtained. Dispensing all three volumes with a 250  $\mu$ L syringe resulted in a RSD of <0.5%, determined gravimetrically. By examining the peak areas after analysis, the dilutions were found to be accurate within 2% (Figure 5).

### Calibration Curve Standard Preparation

Three sets of standards were made both manually and with the Agilent 7696 Sample Prep WorkBench. Comparing the three standard sets on the same plot highlighted the increased reproducibility of the Agilent 7696 Sample Prep WorkBench (Figure 6). While each individual curve yielded  $R^2$  values of 0.999, when plotted together the  $R^2$  value was reduced to 0.934 for the manually prepared standards. In con-

trast, the three curves prepared by the Agilent 7696 Sample prep WorkBench also yielded  $R^2$  values of 0.999 for the individual curves, but when plotted together, the  $R^2$  value was only reduced to 0.997.

Additionally, the relative response factor (RRF) was calculated for each set of standards. Calculating the RSD of the RRFs provides a measure of linearity and reproducibility. The individual calibration curves yielded good RSDs (<5%), demonstrating linear relationships. However, when comparing the three calibration curves together the superiority of the 7696 Sample Prep WorkBench made standards is evident. The average RSD of the RRFs for the three curves made manually was 16%; the three calibration curves made with the 7696 Sample Prep WorkBench gave an average RRF RSD of 4%.

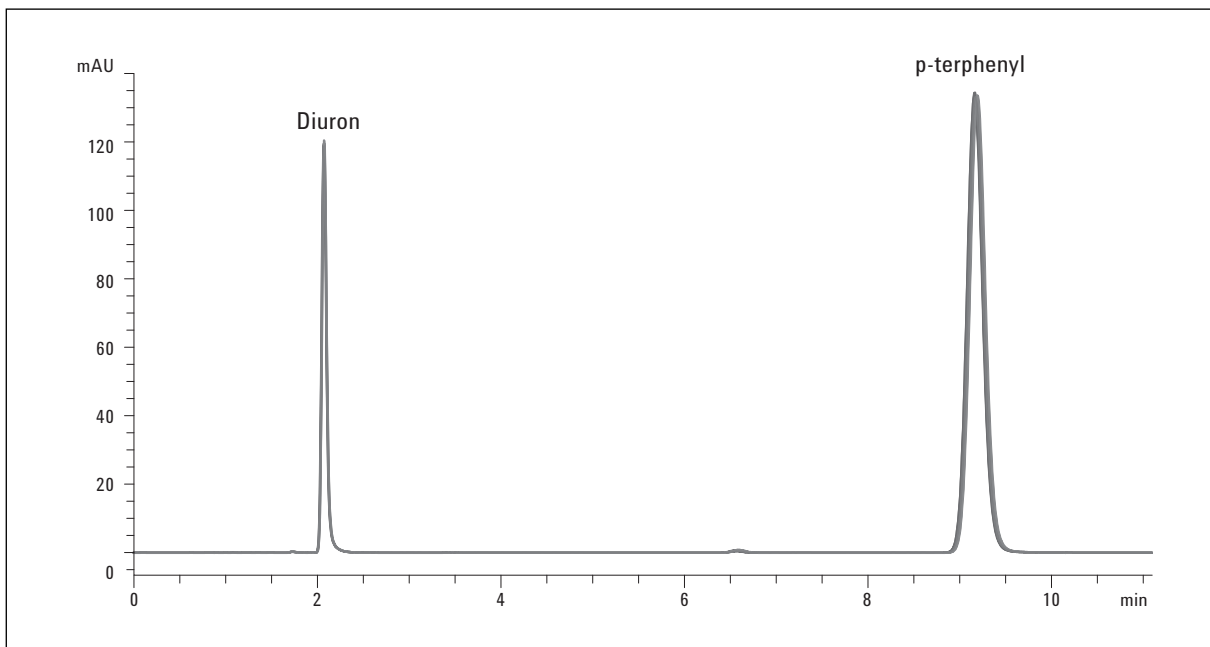


Figure 5. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

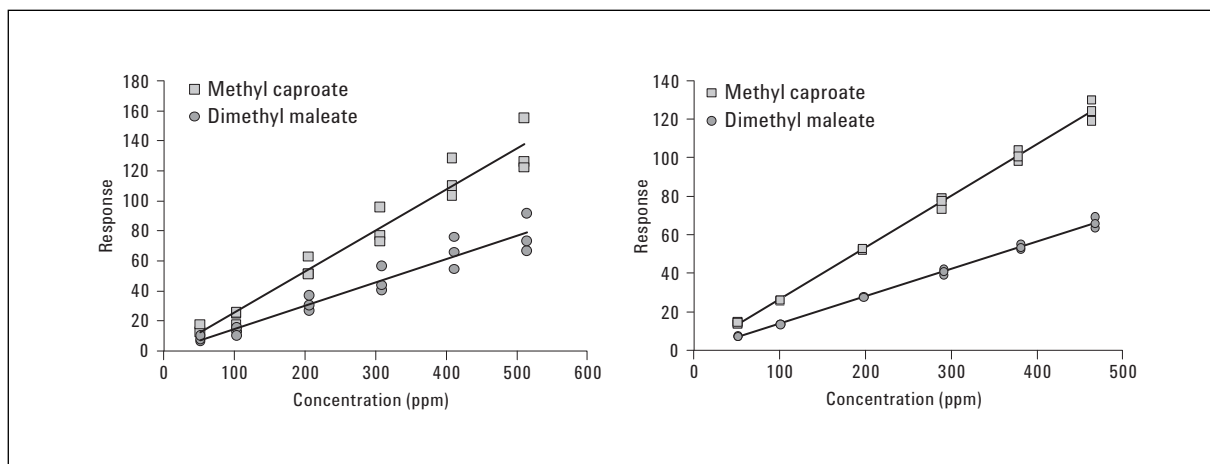


Figure 6. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with the Agilent 7696 Sample Prep WorkBench, are visibly more reproducible than the curves made manually on the left.

## Fatty Acid Derivatization

For sample derivatization, identical results were obtained whether the sample was derivatized manually or with the Agilent 7696 Sample Prep WorkBench. For a set of four fatty acids, no discrimination was observed in either method when derivatizing with a silylating reagent (Table 1). However, as seen with other sample preparation tasks, the Agilent 7696 Sample Prep WorkBench is more reproducible in its liquid delivery. The RSD from the peak areas for the three samples prepared manually 0.9%. The RSD for the three samples prepared with the Agilent 7696 Sample Prep WorkBench was 0.7%.

Table 1. After normalizing the fatty acid peak areas to myristic acid, no discrimination was observed from automating the derivatization

| Analyte       | Ratio-manual | Ratio-automated |
|---------------|--------------|-----------------|
| Capric acid   | 0.92         | 0.92            |
| Capric acid   | 1.2          | 1.2             |
| Myristic acid | 1.0          | 1.0             |
| Palmitic acid | 1.1          | 1.1             |

## Conclusions

The three sample preparation tasks presented in this application note highlight the increased reproducibility achieved by automation with the Agilent 7696 Sample Prep WorkBench. Sample dilutions are accurate and reproducible, calibration curve standards are more linear with fewer errors, and sample derivatizations can be performed without analyte discrimination. However, additional benefits can be reaped through sample prep automation with the Agilent 7696 Sample Prep WorkBench.

By automating calibration curve standard preparation, solvent and reagent usage is significantly reduced. Instead of using >60 mL of solvent to make up standards in 10-mL flasks, only 600 µL of solvent was used, excluding the wash vials. This can result in substantial cost savings for laboratories. Additionally, calibration curve standards required approximately half the time to complete with the Agilent 7696 Sample Prep WorkBench, compared to making up the standards manually. While the other automated sample prep tasks require the same amount of time to complete as the manual methods, the Agilent 7696 Sample Prep WorkBench frees the operator to perform other tasks, such as experiment design or data analysis.

Overall there are many benefits to sample prep automation with the Agilent 7696 Sample Prep WorkBench. While freeing personnel to perform other tasks and reduced solvent usage are important, the largest benefit comes from the reproducibility and accuracy achieved with this system. The automated methods showed better reproducibility and accuracy with fewer errors, thereby improving the quality of the data.

## Reference

1. Susanne Moyer, Dale Synder, Rebecca Veeneman, and Bill Wilson, "Typical Injection Performance for the Agilent 7693A Autoinjector," Agilent Technologies Publication 5990-4606EN.

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