Improved Data Quality Through Automated Sample Preparation

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Introduction

The 7696A Automated Sample Prep WorkBench can perform many sample preparation tasks for either gas chromatographic (GC) or liquid chromatographic (LC) analyses. WorkBench consists of two liquid dispensing modules, a single vial heater capable of reaching 80°C, a single vial vortex mixer, and bar code 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 7693A Automatic Liquid Sampler [1] in an offline setting instead of on top of a GC.

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 preparation, and derivatizations were performed with success on WorkBench. These sample preparation tasks can be time consuming and resource intensive. Automating these procedures with WorkBench can reduce the time and amount of reagents needed and is therefore beneficial in many ways.

Figure 1. The 7696A Sample Prep WorkBench with a gas chromatograph and mass spectrometer (top) and with a liquid chromatograph (bottom).

Experimental

Three common sample preparation tasks were performed with WorkBench.

Sample Dilution and Internal Standard Addition

Sample dilutions and internal standard additions were performed for analysis by both GC and LC. For the GC samples, 50 µL each of isooctane and a standard solution containing four analytes (decane, dodecane, tetradecane, and hexadecane) were added to an empty 2-mL autosampler vial. Additionally 0.5 µL of an internal standard solution (ISTD) containing three analytes (undecane, tridecane, and pentadecane) was added to the vial. The solution was mixed using the onboard vortex 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 µL of acetonitrile, 62.5 µL of a pesticide standard, (diuron) and 125 µL of an ISTD (p-terphenyl) were added. The sample was mixed before being transferred to an LC for analysis. For both of these sample preparations, n=10.

Calibration Curve Standard Preparation

Generic calibration curves for the GC were made in triplicate via linear dilution both manually in 10-mL volumetric flasks and with 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 (methyl valerate, methyl caproate, methyl heptanoate, methyl caprylate, and dimethyl maleate) at 5 mg/mL, ranging from 0.1 to 1 mL, were added using serological pipets. The flasks were diluted to the mark to yield concentrations of 50, 100, 200, 300, 400, and 500 ppm. For the automated method, 100 µL of hexane was added to six empty 2-mL autosampler vials. Again, varying amounts of the stock solution, ranging from 1 to 10 µL, was added to the vials yielding approximately the same concentrations.

By automating calibration curve standard preparation, solvent and reagent usage was significantly reduced. Instead of using >60 mL of solvent to make up standards in 10-mL flasks, only 600 µL of solvent was used for the automated preparation.

Derivatizations

Derivatization of fatty acids via silylation was also performed. For the manual prep, 100 µL of a silylating reagent (BSTFA) was added to approximately 0.5 mL of a free fatty acid solution (caprylic acid, capric acid, myristic acid, and palmitic acid) using an automatic pipettor. The solutions were heated to 70 C using a heated block. The same derivatization was performed with WorkBench using the onboard single vial heater.

Results and Discussion

GC and LC Sample Dilutions

For the samples diluted for GC analysis, the dispensed solvent, standard solution, and ISTD, was measured gravimetrically to determine the reproducibility of the dispensing action. Dispensing 50 µL with a 250 µL syringe resulted in a 0.5% relative standard deviation (RSD) for the 10 samples measured by weight. The samples were diluted accurately within 1%, determined from the peak areas. The ISTD exhibited a slightly lower RSD. Dispensing 0.5 µL with a 25 µL syringe resulted in a RSD of 2% for the 10 samples measured gravimetrically. 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 2).

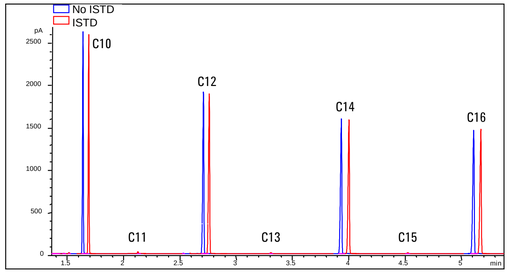

Figure 2. GC chromatograms are shown for a standard solution dispensed and diluted with and without (chromatogram offset) an ISTD added. No difference in the peak areas were observed.

For the samples diluted for LC analysis, similar results were obtained. Dispensing all three volumes with a 250 µL 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 3).

Calibration Curve Standard Preparation

Three sets of standards were made both manually and with WorkBench. Comparing the three standard sets on the same plot highlighted the increased reproducibility achieved when making standards with WorkBench (Figure 4). While each curve yielded R^2 values of 0.999 individually, when plotted together the R^2 value dropped to 0.934 for the manually prepared standards. In contrast, when plotting the standards prepared with WorkBench on a single curve, the R^2 value only dropped to 0.997.

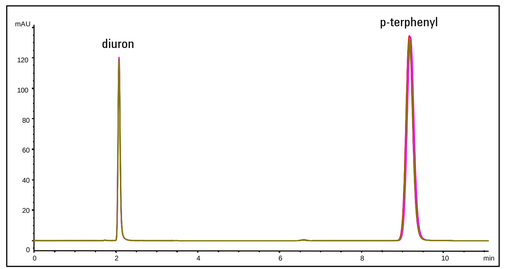

Figure 3. LC Chromatograms are shown for a diluted pesticide standard with an ISTD added. Excellent reproducibility was observed for the five samples shown.

Results and Discussion

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 WorkBench-made standards was evident. The average RSD of the RRFs for the three curves made manually was 16%; the three calibration curves made with WorkBench gave an average RRF RSD of 3.9%.

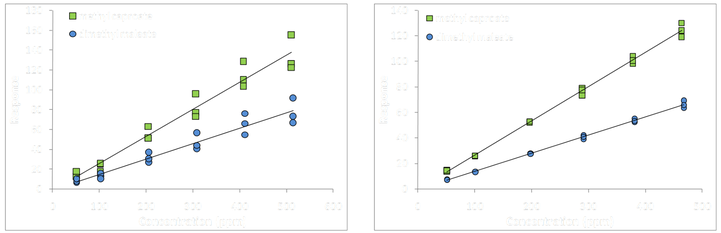

Figure 4. Two calibration curves are shown for two representative analytes. The curves on the right, prepared with WorkBench are visibly more reproducible than the curves made manually on the left.

Fatty Acid Derivatization

For sample derivatization, identical results were obtained whether the sample was derivatized manually or with 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, WorkBench is more reproducible in its solvent/reagent delivery. For three samples prepared manually, an RSD of 0.9% was obtained from the peak areas. For three samples prepared with WorkBench, an RSD of 0.7% was obtained.

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
Caprylic 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 here highlight the increased reproducibility achieved by automating common sample prep tasks with the 7696A 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.

Additional benefits are also achieved. Smaller amounts of solvents/reagents are used and the tasks are often complete in less time. Automating the sample prep also frees personnel to perform other tasks. This can result in substantial cost savings in solvent, glassware, standards, solvent disposal and analyst time for laboratories.

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 overall quality of the data with less need for rework.

References

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