Automated Sample Preparation of Headspace Standards Using the Agilent 7696 WorkBench

Application Note

Forensics and Drug Testing

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Abstract

Preparing a set of calibration standards is useful for determining the amount of analyte in an unknown sample and for establishing the linearity of a system. Manually preparing the standards can be time consuming and susceptible to error. Automated preparation is more time efficient and can avoid potential errors introduced by manual methods. Previous work has demonstrated the use of an Agilent 7696 WorkBench for automating a serial dilution protocol [1]. This article describes the use of the Agilent 7696 WorkBench to successfully prepare a set of blood alcohol calibration standards for analysis by an Agilent 7697 Headspace Sampler. The standards will be prepared in 2-mL vials which will themselves be placed within 20-mL headspace vials. The analysis of the standards prepared by the WorkBench system is compared to the analysis of standards prepared manually. The results show that there is no sacrifice in area precision or system linearity performance from using an automated preparation.

Introduction

Calibration solutions are used extensively in forensic applications such as blood alcohol (BAC) analyses. While a BAC headspace (HS) and gas chromatographic (GC) analysis may be relatively short, the preparation of the necessary calibration standards can be time consuming. By using an automated preparation method a significant time savings can be realized by allowing the user to address other tasks while the samples are being prepared.

The Agilent 7696 WorkBench was designed for automated sample preparation and uses 2-mL vials commonly used in liquid auto-samplers. The 7697 headspace sampler can use 10, 20, and 22-mL headspace vials. A sample volume of ≤ 1 mL is more than sufficient to generate enough usable signal for HS GC analysis. Therefore, the volumes of the calibration standards prepared by the 7696 WorkBench are adequate for headspace analysis. The challenge lies in how to match the two platforms into one analytical solution.

Automating the dilution preparation is beneficial not only for reducing the amount of chemicals that need to be used, since larger volume volumetric glassware is replaced with 2-mL vials, but also for reducing the amount of human intervention and its concomitant potential for user error. The design of the Agilent 7697 headspace and 7696 WorkBench vials are such that a 2-mL 7696 vial can be completely inserted into a 7697 vial. Operation of the 7697 relies on the sampling of the gaseous headspace within each vial, and not on the sample probe directly contacting the sample solution. Placing a 2-mL 7696 vial into a 20-mL 7697 vial ensures that the 7697 probe will not impact the 2-mL vial during sampling, which could potentially damage the probe.

Static headspace analysis involves the sampling of volatile components out of a nonvolatile matrix once the components have reached a thermodynamic equilibrium between the two phases. Heating the sample containing vial increases the amount of volatile analyte in the vial's headspace allowing for better detection response. To decrease the amount of heating time necessary to achieve a given analytical response, headspace vials are often agitated or shaken during heating. The precise control of both sample heating and shaking, along with pneumatic and temporal control, give the 7697 Headspace Sampler industry leading precision and linearity response.

The results described in this application note show that the use of 7696 2-mL vials within 7697 20-mL vials does not compromise the area precision or linearity performance of the 7697 Headspace Sampler. In addition, the results also show that the 7696 WorkBench can be used to accurately prepare calibration standards for headspace applications.

Experimental

Equipment

- Agilent 7696 WorkBench (10-µL syringe, back tower and 500-µL syringe, front tower)
- Agilent 7697 Headspace Sampler, high capacity
- Agilent 7890 Gas Chromatograph
- 250-mL and 100-mL volumetric flasks, ± 0.12 mL and ± 0.08 mL, respectively
- Gilson pipetman pipets

Sample

0.005, 0.01, 0.05, 0.1, and 0.2% each vol/vol: methanol (MeOH), ethanol (EtOH), acetone, isopropanol (IPA), acetonitrile, ethyl acetate, methyl-ethyl ketone (MEK) were made with both manual and automated preparation from a 0.5% stock solution per the steps in Tables 1 and 2, respectively.

The 0.5% stock solution was prepared by adding 1.25 mL of each of the analytes (neat) to a 250-mL volumetric flask and diluting to the mark with water.

For the manual preparation, 1.5-mL aliquots of the final 100-mL solution of each concentration were manually pipeted

Table 1. Manual Sample Preparation Protocol

Concentration %	Vol prepared (mL)	Volume of 0.5% stock	Water (mL)
0.2	100	40	60
0.1	100	20	80
0.05	100	10	90
0.01	100	2.0	98
0.005	100	1.0	99

Table 2. Automated Sample Preparation Protocol

Concentration %	Vol prepared (mL)	Volume of 0.5% stock	Water (mL)
0.2	1.5	0.60	0.90
0.1	1.5	0.30	1.20
0.05	1.5	0.15	1.35
0.01	1.5	0.030	1.47
0.005	1.5	0.015	1.49

into a 20-mL headspace vial and promptly capped. In total, six headspace vials were prepared for each dilution level for a total of 36 vials. For the WorkBench preparation, the 1.5-mL dilution levels prepared for each concentration were kept in the 2-mL vials they were prepared in, but their 2-mL screw caps were removed, and each vial was placed in its own 20-mL headspace vial which was promptly capped after receiving its 2-mL vial. As with the manual prep, six headspace vials (with 2-mL 7696 vials inside) were prepared for each dilution level for a total of 36 vials, therefore the WorkBench method was repeated six times to generate the necessary number of samples.

For each preparation procedure, one dilution series was analyzed sequentially (that is, 0.005% to 0.5%) before any one dilution level was repeated. Two water blanks were run between each dilution series. The headspace and gas chromatograph parameters are given in Table 3:

Table 3. HS and GC Parameters

Agilent 7697 Headspace parameters

Temperatures	Oven = 60 °C, Loop = 60 °C, Tr line = 100 °C
Times	Vial equilibration = 35 min, GC cycle = 6 min, pressure equib. = 0.1 min, Inj = 0.5 min
Vial	Mode = flow-to-pressure, Initial pressure = 15psi, Fill flow = 50 mL/min, Loop fill mode = Custom, Loop fill rate = 20 psi/min, Final loop pressure = 10 psi, Loop equib = 0.05 min, Vial = 20 mL, Shaking = 1
Carrier	GC control
Adv func	Purge flow = 100 mL/min, Purge time = 1 min
Config	Vial pressurization gas type = He, Sample loop volume = 1 mL, Standby flow = 20 mL/min

Agilent 7890 GC parameters

Inlet	S/SI, 5183-4647 liner (wool removed), 200 °C, 33.505 psi, 3 mL/min septum purge, split = 5:1
Columns	DB-ALC2 (J&W 123-9234), 0.320 mm × 30 m, 1.2 µm, constant flow = 12 mL/min
	Dual column configuration for 7696-prepared samples:
	16-cm piece of 530 µm deactivated fused silica (160-2535) connected the inlet to a passive CFT splitter. The splitter was then connected to a DB-ALC2 and a DB-ALC1 column.
	DB ALC1 (J&W 123-9134), 0.320 mm × 30 m, 1.8 µm, constant flow = 12 mL/min
	DB-ALC2 (J&W 123-9234), 0.320 mm × 30 m, 1.2 µm, constant flow = 12 mL/min
Oven	35 °C, hold 4 min
Detector	FID, 250 °C
Signal	FID, 50 Hz (front or back detector)

Results and Discussion

The samples prepared by the Agilent 7696 WorkBench were analyzed with the Agilent 7697 Headspace Sampler using the dual column (DB-ALC1 and DB-ALC2 columns) setup in the Agilent 7890 GC. The manually prepared samples were analyzed using a standard single column (DB-ALC2) setup in the 7890 GC. Representative chromatograms acquired using the dual column system are shown in Figure 1.

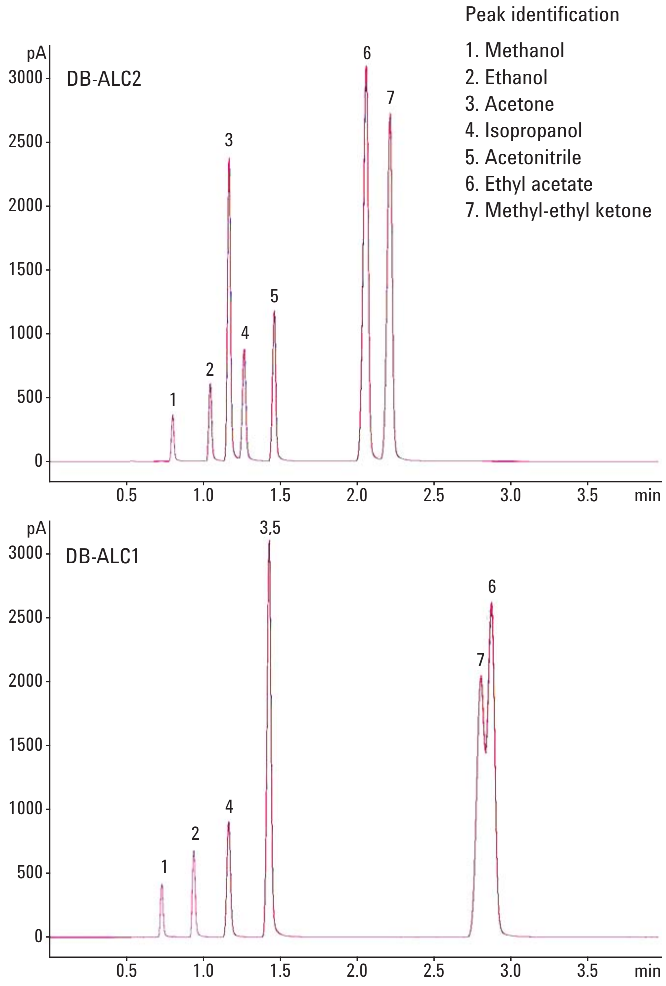

Figure 1. Representative chromatograms of BAC mixture prepared by the Agilent 7696 WorkBench.

The data in Figure 1 represent analytes at the 0.1% concentration level prepared by the 7696 WorkBench. Each chromatogram is actually six overlaid traces from the replicate injections, illustrating the system's precision.

To ensure the HS vials reached thermal equilibrium, the Parameter Increment feature of the Agilent 7697HS Sampler was used to determine at what Vial Equilibration time a constant area response was observed for all analytes. The Vial Equilibration time determination was done using the 0.05% dilution level in an experiment separate from the dilution series evaluation and the results are shown in Figure 2.

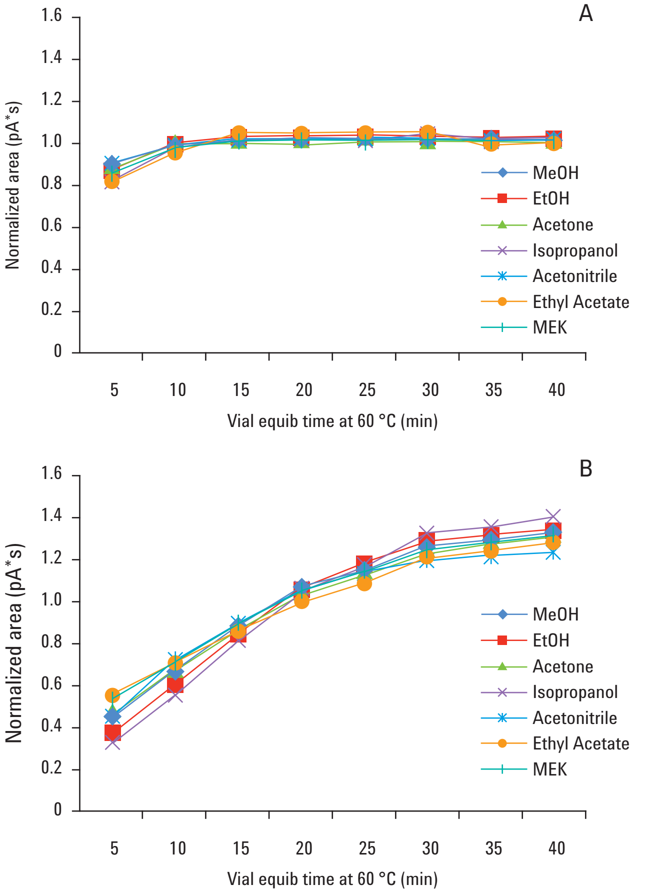

Figure 2. Peak area as a function of Agilent 7697 Headspace Sampler vial equilibration time.
A. 1.5 mL of sample in a 20-mL HS vial. Average of three trials.
B. 1.5 mL of sample in a 2-mL vial within a 20-mL HS vial. Average of three trials.

The experimental results in Figure 2 were used to determine that a 35-min vial equilibration in the 7697 oven was sufficient for the headspace vials containing 2-mL ALS vials. At an equilibration time of 35 min, the area precision that was observed indicated that a thermal equilibrium had been reached. In addition, Figure 2 also shows that a 35-min vial equilibration time was not detrimental to the analysis of headspace vials containing only liquid sample.

Manual Prep

Using a 35-min vial equilibration time, the manually prepared dilution series was analyzed on the 7697 Headspace Sampler using the conditions listed in Table 3. The resulting calibration curve is shown in Figure 3. Table 4 contains the regression values for the manually prepared samples. The results in Figure 3 represent the average areas for six replicate measurements of each concentration level with 1.5 mL of each sample placed directly into the HS vial. Based on the data in Figure 3, the precision for each analyte at each concentration level and the normalization of ethanol to the internal standard, isopropanol, are given in Table 5.

Table 4. Data for Figure 3

Analyte	m	b	R^2
MeOH	26.061	4.0250	0.99999
EtOH	49.950	19.203	0.99998
Acetone	196.08	178.07	0.99963
Isopropanol	83.103	71.332	0.99984
Acetonitrile	101.05	48.276	0.99995
Ethyl acetate	456.69	524.77	0.99945
MEK	373.91	451.77	0.99942

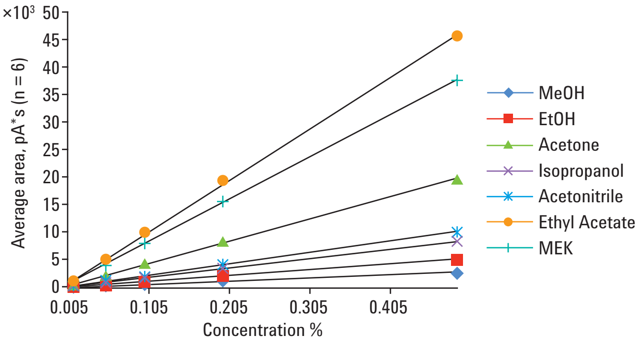

Figure 3. Agilent 7697 Headspace Sampler analysis of manually prepared BAC calibration standards.

The bottom table of Table 5 illustrates the precision of ethanol analysis when an internal standard, in this case, isopropanol is used. The data shows that across the entire calibration range no trending is observed that is unique to any one analyte. For example, if trending was occurring in the ethanol area response then the same magnitude of trending was also occurring with the isopropanol internal standard.

Table 5. Precision of BAC Analysis of Each Manually Prepared Concentration Level

Area precision n = 6

Conc %	MeOH	EtOH	Acetone	Isopropanol	Acetonitrile	Ethyl acetate	MEK
0.005	1.4	1.1	0.54	0.53	0.57	1.8	0.85
0.01	3.3	3.4	1.1	2.2	1.7	1.3	0.89
0.05	1.1	0.93	0.45	0.60	0.53	0.90	0.52
0.1	1.1	0.98	0.46	0.66	0.58	0.89	0.50
0.2	2.4	1.9	0.46	1.1	0.77	0.83	0.54
0.5	1.8	1.8	0.78	1.6	1.2	1.1	0.72

EtOH/IPA ratio

Conc %	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Avg	Stdev	RSD %
0.005	0.56	0.56	0.56	0.57	0.57	0.56	0.56	0.0035	0.62
0.01	0.55	0.55	0.56	0.57	0.56	0.56	0.56	0.0076	1.4
0.05	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.0019	0.34
0.1	0.58	0.58	0.58	0.58	0.59	0.58	0.58	0.0020	0.35
0.2	0.58	0.59	0.59	0.59	0.59	0.59	0.59	0.0051	0.87
0.5	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.0019	0.32

Workbench prep – DB-ALC2

Using a 35-min vial equilibration time, the Agilent 7696 prepared dilution series was analyzed on the Agilent 7697 Headspace Sampler using the conditions listed in Table 3. The calibration curve and its regression values are given in Figure 4 and Table 6 for the analysis run on the DB-ALC2 column. The points in the curves represent the average area response of the six replicate analyses made at each concentration level. The linear regression values show that linearity was maintained across the entire calibration range for all analytes. Table 7 contains the area precision for each analyte at each concentration level and the normalization of ethanol to the internal standard isopropanol for the DB-ALC2 analyses.

Table 6. Linear Regression Results for Automated Sample Preparation from the DB-ALC2 Column

Analyte	m	b	R^2
MeOH	22.545	-7.2643	0.99992
EtOH	41.948	-3.8325	0.99999
Acetone	172.10	-5.8540	0.99997
IPA	66.710	43.059	0.99964
Acetonitrile	91.056	-11.408	0.99999
Ethyl acetate	365.62	-14.323	1.0000
MEK	312.30	90.117	0.99991

The data illustrates a consistent analyte response for different compounds, independent of analyte concentration. The data in the bottom panel of Table 7 again shows that ethanol and isopropanol trend together with respect to area response as a function of concentration, rather than independently. This result shows that the use of the automated sample preparation procedure has not introduced any concentration biases due to erroneous sample handling.

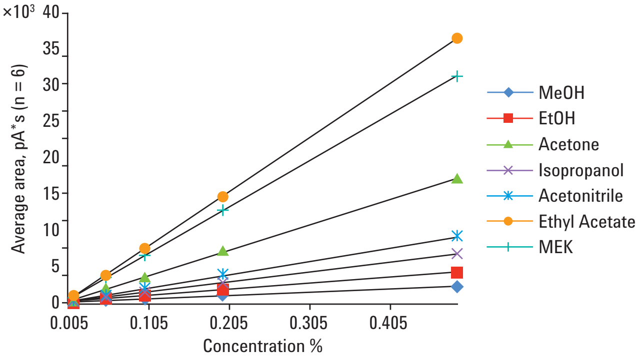

Figure 4. Agilent 7697 Headspace Sampler analysis of BAC standards prepared on the Agilent 7696 WorkBench and analyzed with a DB-ALC2 column.

Table 7. Precision Performance of the Agilent 7697 Headspace Sampler for the Analysis of BAC Standards Prepared on the Agilent 7696 WorkBench and Analyzed with a DB-ALC2 Column

Area precision n = 6

Conc %	MeOH	EtOH	Acetone	Isopropanol	Acetonitrile	Ethyl acetate	MEK
0.005	1.2	1.1	1.5	1.4	1.5	1.8	1.6
0.01	0.52	0.76	0.84	0.70	0.59	1.8	1.1
0.05	0.44	0.50	0.53	0.49	0.37	1.3	0.69
0.1	0.62	0.59	0.58	0.59	0.59	1.0	0.66
0.2	2.2	1.8	0.75	1.2	1.3	1.4	0.83
0.5	0.65	0.56	0.60	0.62	0.48	1.1	0.75

EtOH/IPA ratio

Conc %	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Avg	Stdev	RSD %
0.005	0.57	0.57	0.57	0.57	0.57	0.58	0.57	0.0024	0.43
0.01	0.57	0.58	0.58	0.57	0.58	0.58	0.58	0.0018	0.31
0.05	0.57	0.57	0.57	0.57	0.57	0.58	0.57	0.0014	0.25
0.1	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.0011	0.20
0.2	0.62	0.62	0.62	0.62	0.62	0.61	0.62	0.0048	0.77
0.5	0.63	0.62	0.63	0.62	0.63	0.63	0.63	0.0013	0.20

Workbench Prep – DB ALC1

The results from analyzing the Agilent 7696 prepared dilution series on the Agilent 7697 Headspace Sampler using the DB-ALC1 column under the conditions listed in Table 3 are shown in Figure 5. Due to the nature of the DB-ALC1 column, acetonitrile and acetone are not able to be separated and methyl ethyl ketone and ethyl acetate are not baseline resolved, as observed in Figure 1. The data points in the curves represent the average area response of the six replicate analyses made at each concentration level. The linear regression results are given in Table 8, and again they show that over the range tested, the analytical response for each analyte was linear. Table 9 contains the precision for each analyte at each concentration level and the normalization of ethanol to the internal standard isopropanol for the DB-ALC1 analyses.

Table 8. Linear Regression Results for Automated Sample Preparation from the DB-ALC1 Column

Analyte	m	b	R^2
MeOH	30.515	-13.550	0.99980
EtOH	52.707	-12.505	0.99996
Isopropanol	86.119	-0.42683	1.00000
Acetone/Acetonitrile	316.62	-27.949	1.00000
MEK	442.02	-656.60	0.99807
Ethyl acetate	431.50	555.17	0.99813

Consistent with the previous results, the data in Table 9 shows acceptable raw area precision for each of the compounds. The ethanol area normalized to the iso propanol area displays the same precision as the results from the DB-ALC2 column as well as from the analysis of the manually prepared samples.

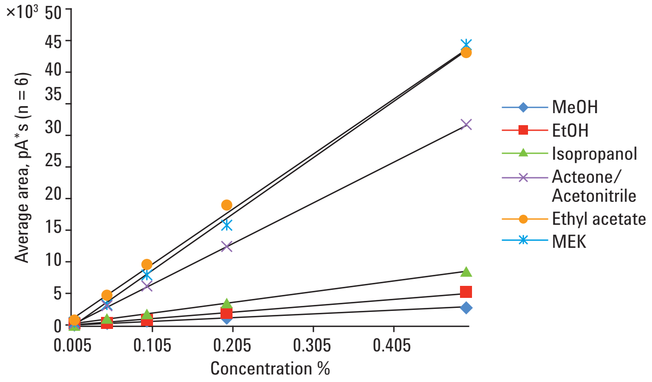

Figure 5. Agilent 7697 Heasdspace Sampler analysis of BAC standards prepared on the Agilent 7696 WorkBench and analyzed with a DB-ALC1 column.

Table 9. Precision Performance of the Agilent 7697 Headspace Sampler for the Analysis of BAC Standards Prepared on the Agilent 7696 WorkBench and Analyzed with a DB-ALC1 Column

Area precision n = 6

Conc %	MeOH	EtOH	Isopropanol	Acetone/Acetonitrile	MEK	Ethyl acetate
0.005	1.2	1.3	1.1	1.4	1.5	1.8
0.01	0.40	0.72	0.73	0.72	1.0	1.8
0.05	0.53	0.42	0.46	0.44	0.69	1.3
0.1	0.52	0.55	0.55	0.53	0.64	1.1
0.2	2.3	1.8	1.2	0.83	0.95	1.5
0.5	0.52	0.54	0.59	0.54	1.0	0.96

EtOH/IPA ratio

Conc %	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Avg	Stdev	RSD %
0.005	0.59	0.60	0.59	0.59	0.59	0.59	0.59	0.0024	0.41
0.01	0.60	0.59	0.59	0.59	0.59	0.60	0.60	0.0016	0.27
0.05	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.0015	0.26
0.1	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.0014	0.23
0.2	0.60	0.60	0.60	0.61	0.60	0.59	0.60	0.0047	0.78
0.5	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.0013	0.20

Summary

The results in Figures 4 and 5 were acquired during the same GC runs using a passive CFT splitter and the dual column arrangement described in Table 3. The dual column configuration produced accurate and precise data and demonstrates the utility of using the Agilent 7696 WorkBench to prepare standards for a common forensics (blood alcohol analysis) application.

To summarize the data presented above, four key metrics have been accumulated in Table 10.

Table 10. Summary Table Comparing Results From Analyzing Samples Prepared Manually to Those Prepared Using the Automated 7696 System

	Concentration %					
EtOH area precision	**0.005**	**0.01**	**0.05**	**0.1**	**0.2**	**0.5**
Manual prep (DB-ALC2)	1.1	3.4	0.93	0.98	1.9	1.8
Automated prep (DB-ALC2)	1.1	0.76	0.50	0.59	1.8	0.56
Automated prep (DB-ALC1)	1.3	0.72	0.42	0.55	1.8	0.54

	Concentration %					
IPA area precision	**0.005**	**0.01**	**0.05**	**0.1**	**0.2**	**0.5**
Manual prep (DB-ALC2)	0.53	2.2	0.6	0.66	1.1	1.6
Automated prep (DB-ALC2)	1.42	0.70	0.49	0.59	1.2	0.62
Automated prep (DB-ALC1)	1.1	0.73	0.46	0.55	1.2	0.59

	Concentration %					
EtOH/IPA ratio precision	**0.005**	**0.01**	**0.05**	**0.1**	**0.2**	**0.5**
Manual prep (DB-ALC2)	0.62	1.4	0.34	0.35	0.87	0.32
Automated prep (DB-ALC2)	0.43	0.31	0.25	0.20	0.77	0.20
Automated prep (DB-ALC1)	0.41	0.27	0.26	0.23	0.78	0.20

Linearity (R^2)	**MeOH**	**EtOH**	**Acetone**	**Isopropanol**	**Acetonitrile**	**Ethyl acetate**	**MEK**
Manual prep (DB-ALC2)	0.99999	0.99980	0.99963	0.99984	0.99995	0.99945	0.99942
Automated prep (DB-ALC2)	0.99992	0.99999	0.99997	0.99964	0.99999	1.00000	0.99991
Automated prep (DB-ALC1)	0.99980	0.99996	not resolved	1.00000	not resolved	0.99813	0.99807

The data in Table 10 shows that for ethanol, isopropanol, and their area ratio the 7696 prepared samples provide more accurate data than the manually prepared alternative except at the lowest concentration level, 0.005%. However, it can be seen in Table 10 that at a concentration of 0.005% the 7696 WorkBench prepared samples still gave area precisions < 1.5% for both ethanol and iso propanol. The linear regression results summarized in Table 10 also illustrate that the linearity performance was unaffected by the type of sample preparation employed or the type of column configuration used.

Conclusion

1. Calibration standards prepared by an Agilent WorkBench demonstrate the same Agilent 7697 Headspace Sampler linearity as manually prepared standards.
2. The same peak area precision was obtained from samples prepared by WorkBench compared to ones prepared manually.
3. The 2-mL vials prepared by WorkBench can be nested within 20-mL headspace vials and successfully analyzed using the 7697 sampler.
4. The nesting of a 2-mL vial within a 20-mL headspace vial will increase the amount of vial equilibration time or agitation needed to achieve a thermodynamic equilibrium between the liquid in the 2-mL vial and the headspace in the 20-mL vial. The impact on equilibration time and agitation level will depend on the method's headspace oven temperature, characteristics of the glass vials, and the properties of the chemical analytes and solvents under test.

References

1. W. Dale Snyder, "Agilent 7696A Sample Prep WorkBench: How to Automate Preparation of a Sample Set by Serial Dilution for Measurement of Flame Ionization Detector Performance", Agilent Application Note, Publication number 5990-6850EN, 2010

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