Generation of Calibration Standards for EPA Method 8082 Using the Agilent 7696A Sample Prep WorkBench

Application Note

Environmental

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Abstract

The Agilent 7696A Sample Prep WorkBench demonstrated precision comparable to the manual method for preparation of calibration standards for the 8802 Method for PCBs. The variation in the average response factor relative standard deviations (RSDs) obtained with the two methods varied less than 0.6%, across the three Aroclor mixes tested. The WorkBench thus provides the necessary reproducibility for this complex analysis without time-consuming hands-on effort and the possibility for human error.

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Introduction

Analytical quality assurance (AQA) is essential for the proper operation of any analytical laboratory, be it commercial, government, or academic. Reliability of data, particularly for analyses regulated by the Environmental Protection Agency (EPA), depends on strict adherence to a wide range of operating procedures for analysis. One of the most common procedures is the use of calibration curves. The accuracy and precision of a quantitation result are completely dependent on the quality of the calibration curves used.

A calibration curve is constructed by preparing a series of standards across a range of concentrations near the expected concentration of analyte in the unknown sample. The deviation of individual calibration points from the line of best fit is used to assess the precision of the calibration. This precision is directly dependent on the quality of the source standard material used as well as the accuracy and reproducibility of the preparation of the calibration standards.

Calibration curves are most commonly prepared manually, involving tedious and time-consuming pipetting steps whose accuracy is dependent on the skill of the operator and the possibility of human error. In addition, the operator may be exposed to hazardous chemicals. Automated dispensing systems remove human error from the process, and assure the accuracy and precision of the preparation of the calibration standards.

This application note demonstrates the utility of the Agilent 7696A Sample Prep WorkBench to automatically prepare calibration standards with comparable or better precision than the same curves derived from manually prepared calibration standards. EPA method 8082 for the detection of polychlorinated biphenyls (PCBs) was used as a model for the demonstration. This method is used to analyze complex mixtures of PCBs called Aroclors that can be a challenge to quantitate reproducibly, using calibration curves.

Both the automated and manual methods gave response factor (RF) relative standard deviations for the calibration curves that were varied across the components of the three Aroclor mixes analyzed. However, with each Aroclor mix WorkBench yielded relative standard deviations (RSDs) which were distributed equally between higher and lower values than those obtained by manually preparing the calibration curves. The average RSDs for the two methods across the three Aroclor mixes were almost identical.

Experimental

Standards and Reagents

Hexane of pesticide grade or higher was used to prepare the calibration standards. PCB Aroclor mix standards, including Aroclor 1248 mix and Aroclor 1016/1260 mix, were obtained from Restek, each at a concentration of 1000 µg/mL. Pesticide surrogate mixes of Tetrachloro-m-xylene (TCMX) and decachlorobiphenyl (DCB) were also obtained from Restek. Two surrogate standards were used with this method to assure acceptable recoveries from matrix after extraction and cleanup. Working solutions of each Aroclor mix standard and the surrogate mix were made up in hexane in 10 µg/mL. These were used to generate calibration standards by dilution into hexane.

Instruments

The calibration standards were prepared on a 7696A Sample Prep WorkBench. The sample analysis was performed on an Agilent 5890 GC System with an Agilent 7673A Autosampler, coupled to an Agilent G1223A Electron Capture Detector. The WorkBench settings are listed in Table 1, and the GC/ECD conditions are listed in Table 2.

Table 1. Agilent 7696A Sample Prep WorkBench Settings

Front liquid handler	500 µL
Back liquid handler	100 µL
Heater setpoint	50 °C
Heater offset	0 °C
Number of pumps	3
Wash volume	400 µL
Draw speed	800 (µL/min)
Dispense speed	2,500 (µL/min)
Draw needle depth offset	0 mm
Viscosity delay	2 seconds
Overfill	5% of syringe size
Air gap	0% of syringe size
Sample processing scheme	Sequential

Table 2. Agilent 5890 GC System with an Agilent G1223A Electron Capture Detector Run Conditions

GC	
Column	30 m × 0.53 mm, 1.5 µm, or 30 m × 0.320 mm, 0.5 µm
Carrier gas	Helium (He)
Column flow	5 mL/min (constant flow 5 psi)
Column temperature program	120 °C hold 0 minutes 9 °C/min to 300 °C Hold 10 minutes Total run time = 30.00 minutes
Make-up gas	Nitrogen (N_2)
Make-up flow	60 mL/min
Injector temperature	250 °C
Detector temperature (ECD)	350 °C
Injection volume	1 µL
ECD	
Make-up gas	N_2

Preparation of Calibration Standards on the Sample Prep WorkBench

Calibration standards were prepared sequentially on the WorkBench. Varying amounts of hexane were first dispensed into vials, then varying amounts of each PCB Aroclor mix standard working solution were dispensed into the same vials to provide a total volume of 1 mL in each vial, and six to eight aroclor calibration standards ranging in concentration from 0.1 to 5 µg/mL. The syringe was rinsed with 400 µL of hexane between each dispensing step.

Results and Discussion

Calibration Curves

The GC analysis of PCB Aroclor mixes (1248 and 1016/1260) results in a very complex chromatogram (Figure 1). Six (1248 and 1016) or eight (1260) of the peaks are quantitated at various retention times and designated as aroclors 1 through 8 (for example, 1260-1 to 1260-8). Using either a manual method or the automated method on the Sample Prep WorkBench, the calibration curve for each of these six to eight aroclors was constructed with an aroclor mix concentration range of 0.1 to 5 µg/mL. Representative calibration curves for Aroclor 1248 using the WorkBench illustrate excellent linearity, with correlation coefficients (R^2) ≥ 0.996 (Figure 2).

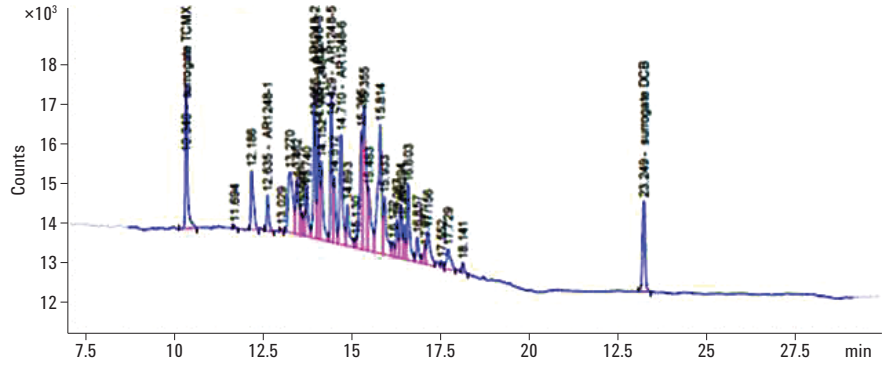

Figure1. Complex GC/ECD chromatogram of Aroclor mix 1248, showing a large number of peaks, six of which were used for the analysis.

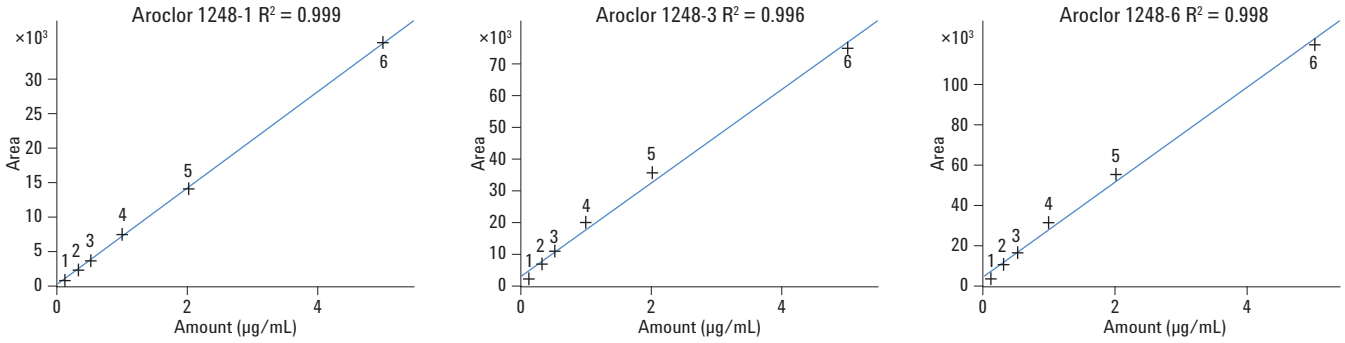

Figure 2. Calibration curves for arocols 1, 3, and 6 of Aroclor 1248 mixture, from 0.1 to 5 µg/mL, prepared using the Agilent 7696A Sample Prep WorkBench.

The average response factors (RFs) across all six concentrations for each aroclor, as well as the standard deviation and relative standard deviation (RSD) are shown in Table 3, upper panel.

The values obtained for Aroclor 1248 from the calibration curves prepared manually were similar to those obtained with calibration standards using the WorkBench automated method. These curves had R^2 values greater than 0.996, but none were greater than 0.998. The average response factors across all six concentrations for each aroclor, as well as the standard deviation and RSD are shown in Table 3, lower panel.

Table 3. Response Factors for Calibration Standards for Aroclors 1–6 of the Aroclor 1248 Mix

WorkBench

Conc (µg/mL)	TCMX	Aroclor-1	Aroclor-2	Aroclor-3	Aroclor-4	Aroclor-5	Aroclor-6	DCB
0.1	1.43215E-06	0.000124348	3.34E-05	4.91E-05	4.94E-05	3.44E-05	3.19E-05	1.57391E-06
0.3	1.25158E-06	0.000130367	3.03E-05	4.37E-05	4.19E-05	3.31E-05	2.84E-05	1.67674E-06
0.5	1.33151E-06	0.000134148	3.23E-05	4.67E-05	4.52E-05	3.46E-05	3.07E-05	1.73147E-06
1	1.41408E-06	0.000135217	3.45E-05	4.94E-05	4.90E-05	3.66E-05	3.22E-05	1.78062E-06
2	1.51853E-06	0.000141773	3.92E-05	5.55E-05	5.64E-05	4.10E-05	3.60E-05	1.87008E-06
5	1.76904E-06	0.00014069	4.78E-05	6.63E-05	6.66E-05	4.90E-05	4.14E-05	2.08277E-06
Ave RF	1.4528E-06	1.3442E-04	3.6241E-05	5.1777E-05	5.1408E-05	3.8113E-05	3.3437E-05	1.7859E-06
Std Dev	1.7970E-07	6.5109E-06	6.3911E-06	8.1325E-06	8.8612E-06	5.9938E-06	4.6388E-06	1.7610E-07
%RSD	**12.37**	**4.84**	**17.64**	**15.71**	**17.24**	**15.73**	**13.87**	**9.86**

Manual

Conc (µg/mL)	TCMX	Aroclor-1	Aroclor-2	Aroclor-3	Aroclor-4	Aroclor-5	Aroclor-6	DCB
0.1	1.10636E-06	8.11997E-05	2.55E-05	3.86E-05	3.92E-05	3.18E-05	3.01E-05	1.25924E-06
0.3	8.95034E-07	0.00010468	2.27E-05	3.39E-05	2.75E-05	2.37E-05	1.97E-05	1.1264E-06
0.5	9.39757E-07	0.00011502	2.51E-05	3.67E-05	2.98E-05	2.66E-05	2.18E-05	1.0728E-06
1	1.03707E-06	0.000118838	2.92E-05	4.16E-05	3.70E-05	3.04E-05	2.58E-05	1.09724E-06
2	1.15416E-06	9.96355E-05	3.16E-05	4.45E-05	4.03E-05	3.29E-05	2.75E-05	1.34211E-06
3	1.24793E-06	0.000103842	3.41E-05	4.78E-05	4.42E-05	3.52E-05	2.94E-05	1.28206E-06
Ave RF	1.0634E-06	1.0387E-04	2.8028E-05	4.0504E-05	3.6329E-05	3.0115E-05	2.5727E-05	1.1966E-06
Std Dev	1.3301E-07	1.3273E-05	4.3373E-06	5.1628E-06	6.4287E-06	4.2278E-06	4.1752E-06	1.1183E-07
%RSD	**12.51**	**12.78**	**15.47**	**12.75**	**17.70**	**14.04**	**16.23**	**9.35**

The two surrogate standards (TCMX and DCB) are also shown.

Ave RF = average response factor

Std Dev = standard deviation

RSD = relative standard deviation

Representative calibration curves for Aroclor mixes 1016 and 1260 using the WorkBench illustrate good linearity, with $R^2 \geq 0.996$ (Figures 3 and 4).

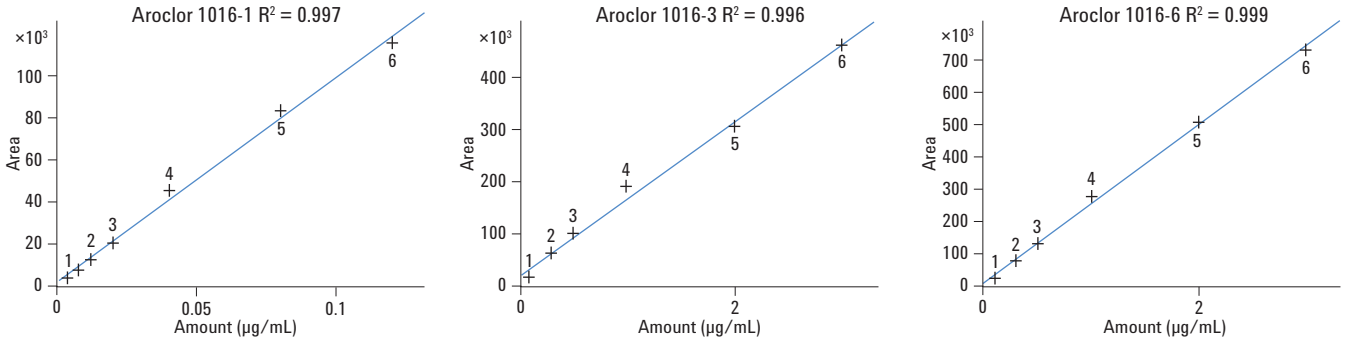

Figure 3. Calibration curves for aroclors 1, 3, and 6 of Aroclor 1016 mixture, from 0.1 to 5 µg/mL, prepared using the Agilent 7696A Sample Prep WorkBench.

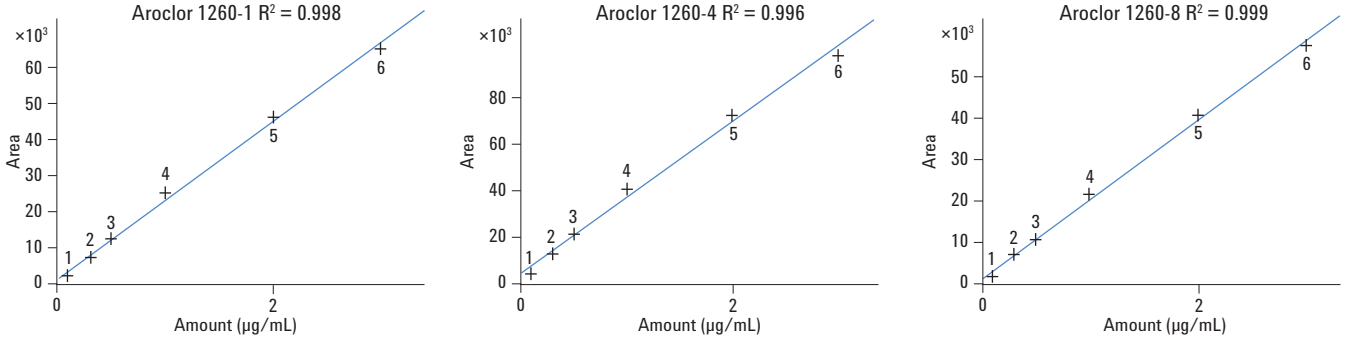

Figure 4. Calibration curves for aroclors 1, 4, and 8 of Aroclor 1260 mixture, from 0.1 to 5 µg/mL, prepared using the Agilent 7696A Sample Prep WorkBench.

The average response factors (RFs) across all six concentrations for each aroclor, as well as the standard deviation and RSD are shown in Table 4.

Table 4. Response Factors for WorkBench Calibration Standards for Aroclors 1–6 and 1–8 of the Aroclor Mixes 1016 and 1260

WorkBench

1016

Conc (µg/mL)	TCMX	Aroclor-1	Aroclor-2	Aroclor-3	Aroclor-4	Aroclor-5	Aroclor-6	DCB
0.1	1.09445E-06	2.74456E-05	2.28849E-05	5.75766E-05	5.71135E-05	5.81004E-05	4.10239E-05	1.44807E-06
0.3	9.52E-07	2.78E-05	1.99E-05	4.82E-05	4.60E-05	4.60E-05	3.83E-05	1.45E-06
0.5	9.68E-07	3.10E-05	2.04E-05	4.97E-05	4.58E-05	4.50E-05	3.81E-05	1.47E-06
1	8.8216E-07	3.08E-05	2.07E-05	5.24E-05	4.42E-05	4.20E-05	3.60E-05	1.47E-06
2	9.57521E-07	3.40E-05	2.87E-05	6.52E-05	4.91E-05	4.77E-05	3.97E-05	1.57E-06
5	1.03499E-06	3.71E-05	2.57E-05	6.49E-05	5.18E-05	4.48E-05	4.14E-05	1.72E-06
Ave RF	9.8146E-07	3.1362E-05	2.3043E-05	5.6327E-05	4.9005E-05	4.7284E-05	3.9079E-05	1.5222E-06
Std Dev	7.3673E-08	3.7056E-06	3.5002E-06	7.4917E-06	4.8076E-06	5.6146E-06	2.0134E-06	1.0563E-07
%RSD	**7.51**	**11.82**	**15.19**	**13.30**	**9.81**	**11.87**	**5.15**	**6.94**

1260

Conc (µg/mL)	Aroclor-1	Aroclor-2	Aroclor-3	Aroclor-4	Aroclor-5	Aroclor-6	Aroclor-7	Aroclor-8
0.1	4.40825E-05	1.88138E-05	1.61092E-05	2.13873E-05	2.50066E-05	3.08609E-05	5.81754E-05	5.82256E-05
0.3	3.93E-05	1.84E-05	1.73E-05	2.30E-05	2.93E-05	2.66E-05	4.18E-05	4.28E-05
0.5	3.96E-05	1.87E-05	1.73E-05	2.30E-05	2.73E-05	3.11E-05	5.18E-05	4.72E-05
1	3.95E-05	1.95E-05	1.78E-05	2.43E-05	2.87E-05	3.06E-05	5.10E-05	4.64E-05
2	4.36E-05	2.21E-05	1.98E-05	2.75E-05	3.21E-05	3.25E-05	5.48E-05	4.92E-05
5	4.61E-05	2.44E-05	2.17E-05	3.04E-05	3.54E-05	3.55E-05	6.02E-05	5.23E-05
Ave RF	4.2033E-05	2.0320E-05	1.8331E-05	2.4937E-05	2.9643E-05	3.1183E-05	5.2961E-05	4.9342E-05
Std Dev	2.9364E-06	2.3906E-06	2.0451E-06	3.3799E-06	3.6551E-06	2.8742E-06	6.5123E-06	5.3518E-06
%RSD	**6.99**	**11.76**	**11.16**	**13.55**	**12.33**	**9.22**	**12.30**	**10.85**

The two surrogate standards (TCMX and DCB) are also shown.

Ave RF = average response factor

Std Dev = standard deviation

RSD = relative standard deviation

The RF %RSD values obtained for Aroclor mixes 1016 and 1260 from the calibration curves prepared manually were similar to those obtained with calibration standards using the WorkBench automated method, with $R^2 \geq 0.996$. The average response factors across all six concentrations for each aroclor, as well as the standard deviation and RSD are shown in Table 5.

Table 5. Response Factors for Manual Calibration Standards for Aroclors 1–6 and 1–8 of the Aroclor Mixes 1016 and 1260

Manual				**1016**				
Conc (µg/mL)	**TCMX**	**Aroclor-1**	**Aroclor-2**	**Aroclor-3**	**Aroclor-4**	**Aroclor-5**	**Aroclor-6**	**DCB**
0.1	1.11553E-06	3.64871E-05	2.39579E-05	8.11532E-05	5.45518E-05	0.000054662	4.80671E-05	1.58306E-06
0.3	1.28E-06	4.01E-05	2.44E-05	5.12E-05	5.72E-05	5.65E-05	4.80E-05	1.52E-06
0.5	1.29E-06	4.00E-05	2.57E-05	5.85E-05	5.83E-05	5.83E-05	4.94E-05	1.54E-06
1	1.32786E-06	4.50E-05	2.93E-05	6.74E-05	6.30E-05	5.75E-05	5.17E-05	1.77E-06
2	1.3179E-06	4.57E-05	3.10E-05	7.99E-05	6.50E-05	6.26E-05	5.31E-05	1.77E-06
5	1.40561E-06	4.84E-05	3.35E-05	8.36E-05	6.72E-05	6.48E-05	5.49E-05	1.88E-06
Ave RF	1.2897E-06	4.2602E-05	2.7978E-05	7.0284E-05	6.0885E-05	5.9074E-05	5.0861E-05	1.6740E-06
Std Dev	9.6034E-08	4.4550E-06	3.8891E-06	1.3422E-05	4.9413E-06	3.8567E-06	2.8387E-06	1.4853E-07
%RSD	**7.45**	**10.46**	**13.90**	**19.10**	**8.12**	**6.53**	**5.58**	**8.87**

				1260				
Conc (µg/mL)	**Aroclor-1**	**Aroclor-2**	**Aroclor-3**	**Aroclor-4**	**Aroclor-5**	**Aroclor-6**	**Aroclor-7**	**Aroclor-8**
0.1	5.78262E-05	2.29653E-05	1.91661E-05	3.30329E-05	3.67605E-05	4.15603E-05	8.89391E-05	7.69059E-05
0.3	4.86E-05	2.03E-05	1.72E-05	2.80E-05	3.21E-05	3.74E-05	7.68E-05	7.15E-05
0.5	5.03E-05	2.19E-05	1.87E-05	3.07E-05	3.56E-05	3.77E-05	7.66E-05	7.13E-05
1	5.81E-05	2.67E-05	2.30E-05	3.91E-05	4.62E-05	4.33E-05	8.80E-05	8.07E-05
2	5.85E-05	2.78E-05	2.33E-05	3.93E-05	4.47E-05	4.26E-05	8.69E-05	7.90E-05
5	5.85E-05	2.97E-05	2.47E-05	4.22E-05	4.75E-05	4.42E-05	7.08E-05	8.19E-05
Ave RF	5.5295E-05	2.4908E-05	2.0984E-05	3.5391E-05	4.0480E-05	4.1126E-05	8.1343E-05	7.6899E-05
Std Dev	4.5364E-06	3.7082E-06	3.0355E-06	5.6087E-06	6.4463E-06	2.9098E-06	7.5718E-06	4.5555E-06
%RSD	**8.20**	**14.89**	**14.47**	**15.85**	**15.92**	**7.08**	**9.31**	**5.92**

The two surrogate standards (TCMX and DCB) are also shown.

Ave RF = average response factor

Std Dev = standard deviation

RSD = relative standard deviation

WorkBench versus Manual Preparation

The striking result of a comparison of the RSDs in this demonstration is the strong similarity of the WorkBench and manual methods, in spite of the complex nature of the Aroclor mixes. For the components of each Aroclor mix, approximately half of the RSDs were lower using the WorkBench, and half were lower using manual preparation (Tables 3–4). While in a few cases these differences were large (for example, aroclor-1 of Aroclor 1248 mix), average RSDs calculated across all the aroclor components of all three Aroclor mixes varied less than 0.6% across all three Aroclor mixes (Table 5). Clearly, the Sample Prep WorkBench is a viable alternative for preparation of calibration standards, yielding nearly identical precision to manual preparation, without tedious and time-consuming hands-on effort.

Conclusion

Preparation of accurate and precise calibration standards is an absolute necessity for every analytical laboratory. This is particularly true for EPA methods in environmental laboratories. Method 8082 is challenging due to the complex nature of the PCB mixes and the resulting complex chromatograms. The Agilent Sample Prep WorkBench can provide precision comparable to the manual method for preparation of calibration standards, thus providing necessary reproducibility for a complex analysis without time-consuming hands-on effort and the possibility for human error.

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Table 5. Comparison of the Average Response Factor RSDs Obtained Across all Aroclors of all Three Aroclor Mixes, Using the Agilent 7696A Sample Prep WorkBench and Manual Preparation of Calibration Standards

Aroclor mix calibration standard preparation method	1248		1016		1260	
	WorkBench	Manual	WorkBench	Manual	WorkBench	Manual
Average %RSDs	13.41	13.85	10.57	10.00	11.02	11.46

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