The Advantages of Automated Sample Preparation

Application Note

Author

Anne Jurek
Applications Chemist
EST Analytical
Cincinnati, OH

Abstract

Sample preparation is one of the most important steps in analytical chemistry. Attention to detail and accuracy are essential. For these reasons, many laboratories are interested in automating sample preparation procedures so as to limit the possibility of human error. Furthermore, quantitative analysis requires not only sample preparation but also standard preparation. When making a calibration curve, the analyst needs to follow a recipe in order to ensure standard accuracy. Any mistake in the standard formulation and the calibration curve will need to be re-prepped and re-run. Thus, valuable time and resources are wasted. This application will explore automated standard preparation of Poly Aromatic Hydrocarbon (PAH) compounds.

Introduction:

Many laboratories have procedures in place for standard and/or sample preparation. Often times these procedures can be not only tedious, but also time consuming. For this reason, automated sample preparation can be an invaluable tool. Furthermore, human error can be eliminated because, once an automated procedure is established, the preparation of samples will be executed in the same manner every time.

This application note will demonstrate the automated preparation of a seven point Poly Aromatic Hydrocarbon calibration curve. The automated preparation will then be validated by running the calibration curves on a Gas Chromatograph/Mass Spectrometer (GC/MS). The calibration preparation and curve validation will be repeated four times in order to display the reproducibility and accuracy of the sample preparation. Furthermore, each calibration curve will be used to calculate the recovery of one standard in order to compare the precision of each calibration curve.

Experimental:

The FLEX Series autosampler was utilized for the automated sample preparation. Semi-volatile Calibration mix #5 PAH standard was ordered from Restek. Next, 20μg/mL and 500μg/mL standards were prepared and placed in the 2mL sample tray of the FLEX. Finally, a 500μl syringe was installed on the FLEX and the experimental parameters were set to prepare the calibration curve. See Tables 1 and 2.

Autosampler	Flex
General	
Method Type	Liquid
Sample Preparation (For Diluent 1 and Diluent 2)	
Solvent Source	10mL Solvent/Waste 1
Solvent Location	3
Solvent Needle Depth	95%
Solvent Needle Depth Speed	60%
Solvent Pump Cycles	2
Solvent Dispense Rate	75%
Solvent Pump Volume	100 to 110% of Solvent Volume
Solvent Volume	See Standard Preparation Table Diluent 1 and 2
Solvent Fill Rate	2%
Solvent Fill Delay	1 sec
Sample Vial Needle Depth	85%
Sample Preparation	
Solvent Source	2mL Tray
Solvent Location	99 (20μg/mL) or 100 (500μg/mL)
Solvent Needle Depth	90%
Solvent Needle Depth Speed	60%
Solvent Pump Cycles	1
Solvent Dispense Rate	75%
Solvent Pump Volume	100 to 110% of Solvent Volume
Solvent Volume	See Standard Preparation Table Source Standard Volume
Solvent Fill Rate	1%
Solvent Fill Delay	1 sec
Sample Vial Needle Depth	85%
Sample	
Sample Vial Depth	90%
Sample Vial Depth Speed	60%
Sample Volume	0µL
Sample Fill Rate	5%
Sample Pump Volume	80%
Dispense Rate	100%
Pump Cycles	2
Rinse	
Rinse/Waste Type	10mL Solvent/Waste 1
Rinse Position	1
Waste Position	2
Rinse Volume	100% (500μL)
Rinse Fill Rate	5%
Rinse Cycles	2
Rinse Dispense Rate	100%
Rinse Depth	90%
Rinse Depth Speed	80%
Waste Depth	50%

Table 1: FLEX Autosampler Sample Preparation Experimental Parameters

Cal Level	Std. Conc. (µg/mL)	Std. Source Conc. (µg/mL)	Diluent 1 Solvent Vol. (µL)	Diluent 2 Solvent Vol. (µL)	Source Standard Vol. (µL)	Final Vol. (µL)
1	1	20	500	450	50	1000
2	5	20	500	250	250	1000
3	10	20	500	0	500	1000
4	25	500	500	450	50	1000
5	50	500	500	400	100	1000
6	100	500	500	300	200	1000
7	200	500	500	100	400	1000

Table 2: Calibration Curve Preparation Volumes

After the calibration curve was prepared, the accuracy of the sample preparation needed to be verified. Thus, a 10μl syringe was mounted on the FLEX and the FLEX was then configured to run with an Agilent 7890 GC/5975MS. A Restek Rxi-5ms 30m x 0.25mm x 0.25μm column was fitted in the GC for compound separation. Tables 3 and 4 display the sampling and analysis experimental parameters.

Autosampler	FLEX
General	
Method Type	Liquid
Rinse	
Rinse/Waste Type	10mL Solvent/Waste 1
Rinse Position	1
Waste Position	2
Rinse Volume	50% (5μL)
Rinse Fill Rate	10%
Rinse Cycles	2
Rinse Dispense Rate	100%
Rinse Depth	90%
Rinse Depth Speed	50%
Waste Depth	50%
Sample	
Sample Vial Depth	95%
Sample Vial Depth Speed	50%
Sample Volume	10% (1μL)
Sample Fill Rate	5%
Sample Fill Delay	1sec
Rinse Volume	50% (5μL)
Rinse Cycles	1
Sample Pump Volume	50% (5μL)
Dispense Rate	100%
Pump Cycles	3
Air Volume Gap	
Air Fill Volume	10% (1μl)
Single Injection Port	
Needle Depth	90%
Injection Rate	100%
Injection Volume	20% (2μl)
Pre-Injection Delay	1 sec
Post Injection Delay	1 sec
Rinse (Repeat Rinse Step Above)	

Table 3: FLEX Autosampler Injection Experimental Parameters

GC/MS	Agilent 7890/5975 (10µl)
Inlet	Split/Splitless
Inlet Temp.	250ºC
Inlet Head Pressure	13.312 psi
Split	20:1
Liner	Gooseneck Splitless Liner, 4mm x 6.5 x 78.5 with deactivated wool
Column	Rxi-5Sil MS 30m x 0.25mm I.D. x 0.25µm film thickness
Oven Temp. Program	40ºC hold for 0.5 min, ramp 10ºC/min to 100ºC hold for 0.0 min, ramp 25ºC/min to 260ºC hold for 0.0 min, ramp 5ºC/min to 280ºC hold for 0.0 min, ramp 15ºC/min to 320ºC hold for 2.0 min, 21.5 min run time
Column Flow Rate	1.2mL/min.
Gas	Helium
Total Flow	28.2mL/min.
Source Temp.	230ºC
Quad Temp.	150ºC
MS Transfer Line Temp.	280ºC
Solvent Delay	2.5 min
Acquisition Mode	Scan
Scan Range	m/z 35-500
Sampling Rate	3.12 scans/sec

Table 4: GC/MS Experimental Parameters

The Four calibration curves prepared by the FLEX autosampler were evaluated for linearity. The Ave. %RSD linearity of the PAH compounds are listed in Table 5. Table 6 displays the accuracy and reproducibility of the automated sample preparation by calculating the recovery of all the PAH compounds using each individual curve and one 50ng sample injection. Finally, a 50ng standard chromatogram is displayed below, see Figure 1.

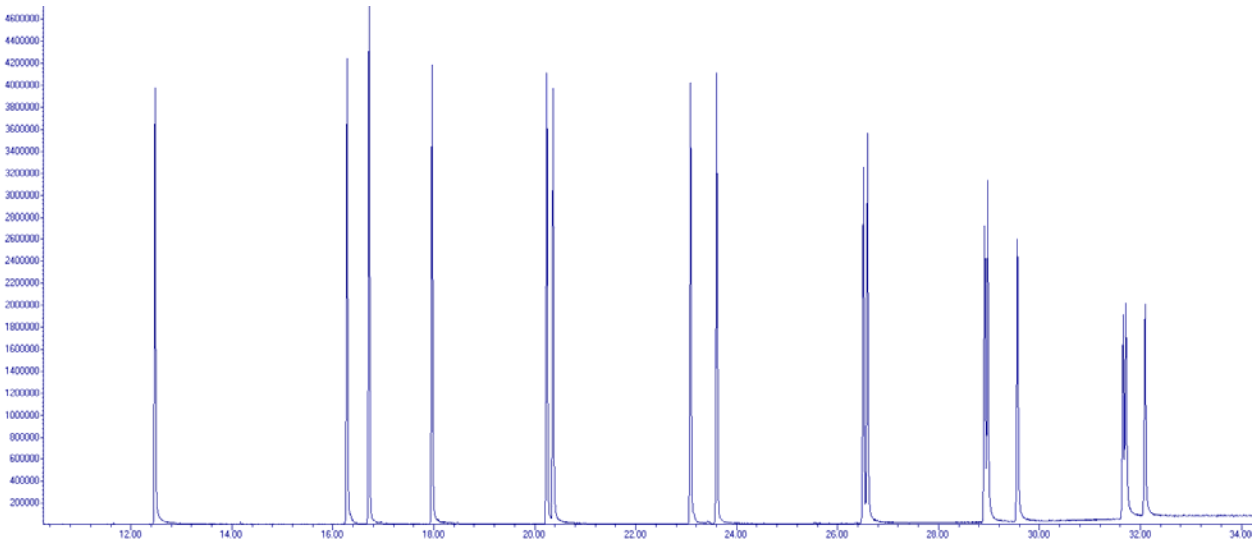

Figure 1: Chromatogram of 50ng/µl PAH Standard

Compound	Calibration Curve %RSD				
	1	2	3	4	Ave. %RSD
Naphthalene	13.17	11.03	10.74	11.65	11.65
Acenaphthalene	9.07	6.95	7.70	8.11	7.96
Acenaphthene	7.44	5.25	4.61	8.92	6.56
Fluorene	9.26	5.99	7.77	8.39	7.85
Phenanthrene	7.76	6.54	10.76	11.23	9.07
Anthracene	5.36	4.13	7.20	8.01	6.18
Fluoranthene	6.15	7.67	10.23	12.40	9.11
Pyrene	8.93	11.47	8.61	5.61	8.66
Benz(a)anthracene	12.71	13.84	18.94	15.50	15.25
Chrysene	9.73	6.27	8.20	8.47	8.17
Benzo(b)fluoranthene	13.79	14.98	20.00	13.66	15.61
Benzo(k)fluoranthene	8.32	6.86	7.37	10.32	8.22
Benzo(a)pyrene	8.28	9.69	13.11	13.66	11.19
Indeno(1,2,3-cd)pyrene	13.11	15.99	16.66	17.91	15.92
Dibenz(a,h)anthracene	11.49	12.99	13.13	13.40	12.75
Benzo(g,h,i)perylene	7.34	6.73	10.80	9.69	8.64

Table 5: Linearity of PAH compounds in %RSD of Response Factors

Compound	50ng Sample Results							
	Curve 1	Curve 2	Curve 3	Curve 4	Ave.	Std. Dev.	%RSD	%Rec'ry
Naphthalene	47.39	46.77	46.69	47.37	47.06	0.33	0.69	94.11
Acenaphthalene	45.90	45.58	44.71	46.53	45.68	0.66	1.44	91.36
Acenaphthene	45.05	44.64	43.19	44.14	44.26	0.69	1.57	88.51
Fluorene	46.41	45.70	44.54	46.11	45.69	0.71	1.55	91.38
Phenanthrene	47.06	46.10	46.91	48.06	47.03	0.70	1.48	94.07
Anthracene	45.38	43.89	44.52	45.98	44.94	0.80	1.78	89.89
Fluoranthene	44.78	45.07	44.63	45.64	45.03	0.39	0.86	90.06
Pyrene	43.38	42.95	45.43	46.71	44.62	1.53	3.43	89.24
Benz(a)anthracene	46.51	47.19	49.77	51.44	48.73	1.98	4.07	97.46
Chrysene	46.58	44.54	46.29	47.71	46.28	1.14	2.45	92.56
Benzo(b)fluoranthene	45.75	47.27	48.20	50.18	47.85	1.60	3.35	95.70
Benzo(k)fluoranthene	47.14	44.73	45.79	46.62	46.07	0.91	1.98	92.14
Benzo(a)pyrene	42.68	43.65	45.08	47.75	44.79	1.91	4.27	89.58
Indeno(1,2,3-cd)pyrene	49.37	43.73	43.90	50.21	46.80	3.00	6.42	93.61
Dibenz(a,h)anthracene	42.46	46.11	45.61	47.19	45.34	1.76	3.88	90.69
Benzo(g,h,i)perylene	41.98	42.92	42.90	44.25	43.01	0.81	1.88	86.03
Ave.							2.57	91.65

Table 6: Precision of Each Curve Using a 50ng Standard

Conclusions:

The FLEX Series autosampler, with its innovative software, made implementing an automated curve preparation routine both simple and intuitive. Once the method routines were inputted, the FLEX did all of the work. The results of the automated curves met USEPA Method 8270 requirements and when testing the calibration curves against a mid-range calibration standard, all of the calibration curves displayed very similar results. In fact, the percent relative standard deviation for the four calibration curves compound results were averaged to be less than three percent while the average recovery was over 90%. The FLEX Series autosampler would make an excellent addition to any lab wishing to optimize their time and money by automating sample preparation.

References:

1. Method 8270D, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), USEPA, Revision 4, February 20007.

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