



Sample name: **M18**  
Assay name: **UV-metric pKa**  
Assay ID: **17J-03026**  
Filename: **C:\Sirius\_T3\17J-03026\_M18\_UV-metric pKa.t3r**

Experiment start time: **10/3/2017 7:54:42 PM**  
Analyst: **Dorothy Levorse**  
Instrument ID: **T311053**

## Results

pKa 1 **5.37**  
pKa 2 **10.65**  
RMSD **0.007 0.003 0.004**  
Chi squared **0.1865**  
PCA calculated number of pKas **3**  
Average ionic strength **0.158 M**  
Average temperature **24.9°C**  
Analyte concentration range **48.1 µM to 43.4 µM**

Number of pKas source **Predicted**  
Wavelength clipping **230.0 nm to 450.0 nm**  
pH clipping **1.270 to 12.739**

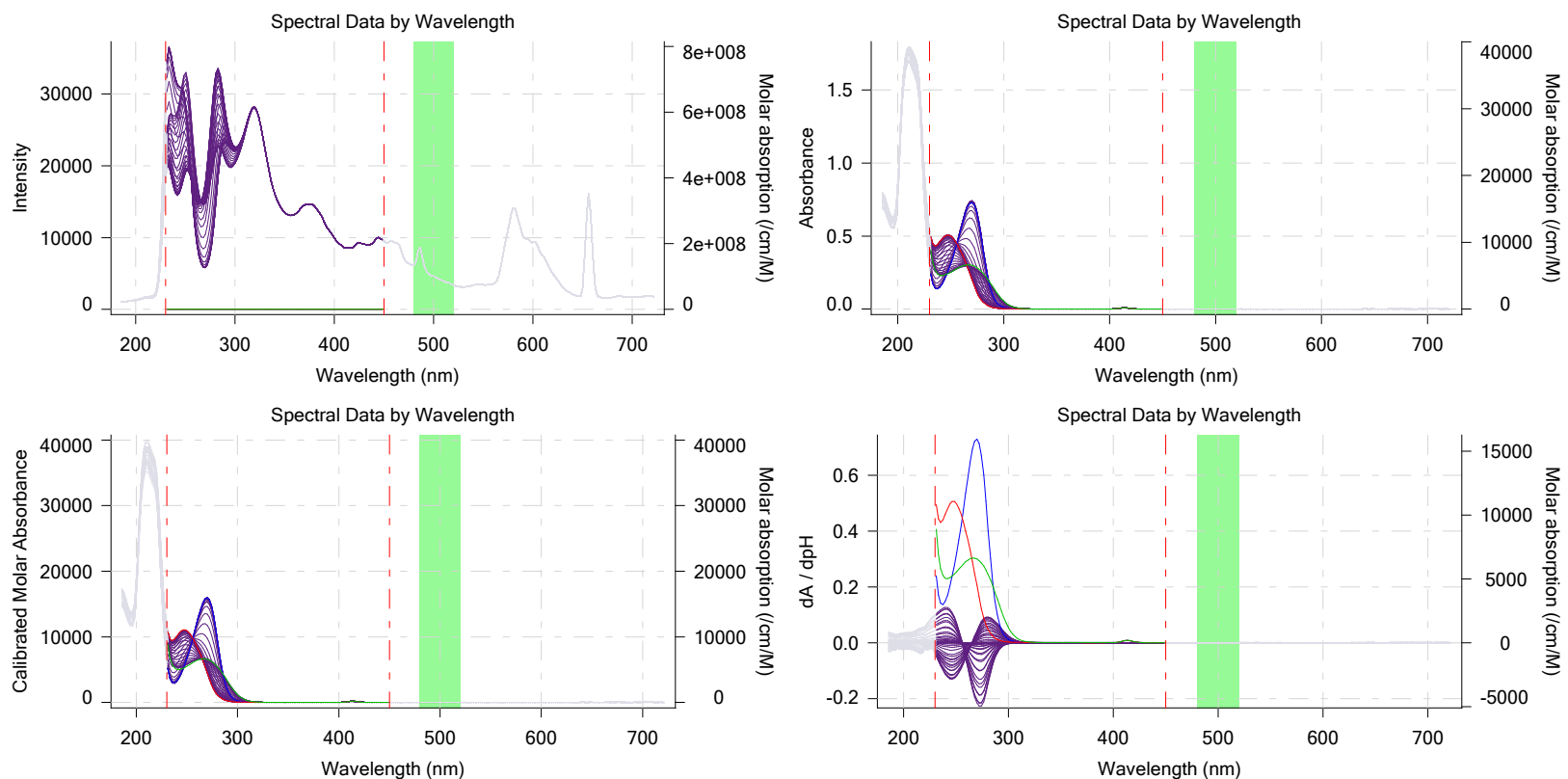
## Warnings and errors

Errors **None**  
Warnings **PCA calculation disagrees with predicted number of pKas**

## Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
<b>Assay Medium</b>				
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			

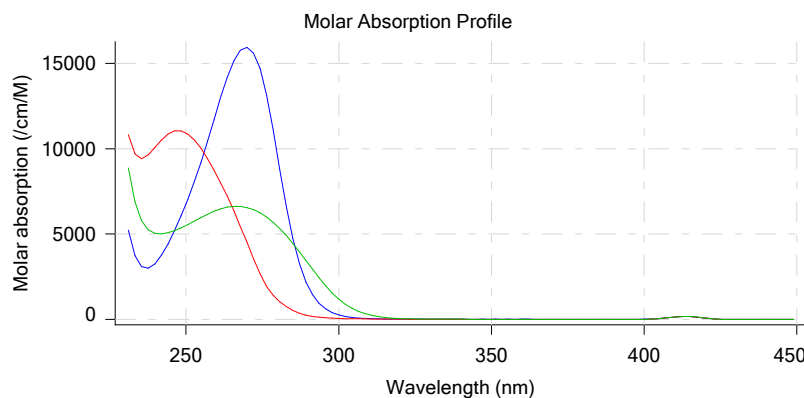
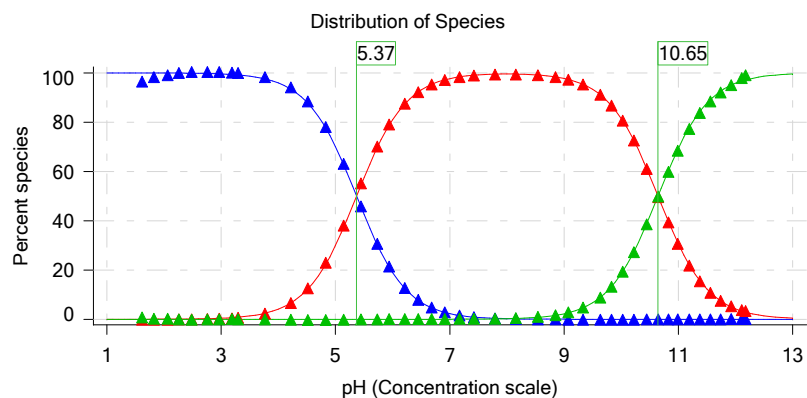
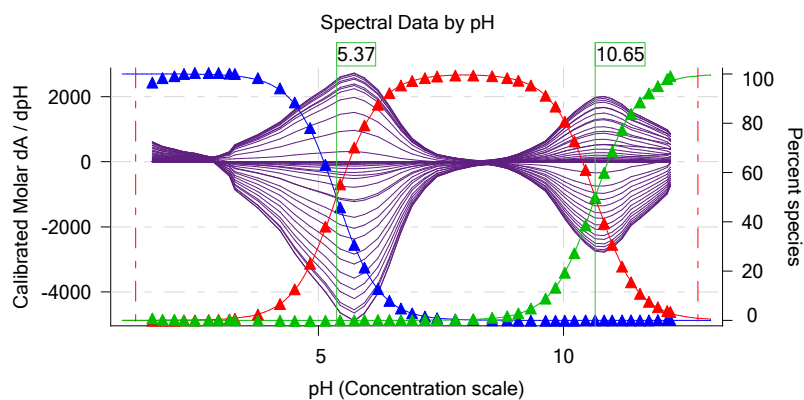
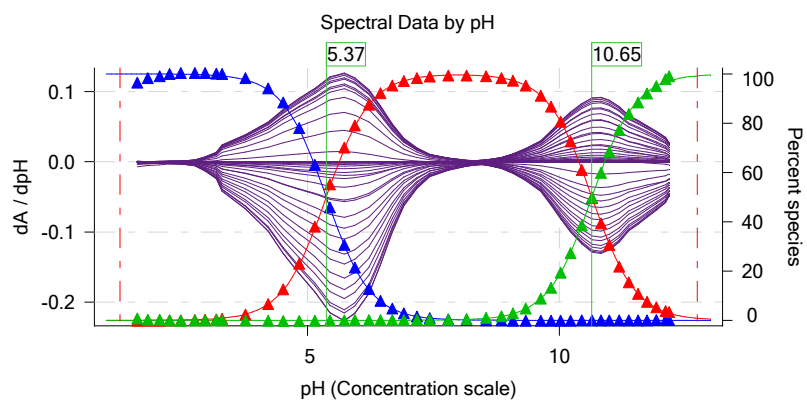
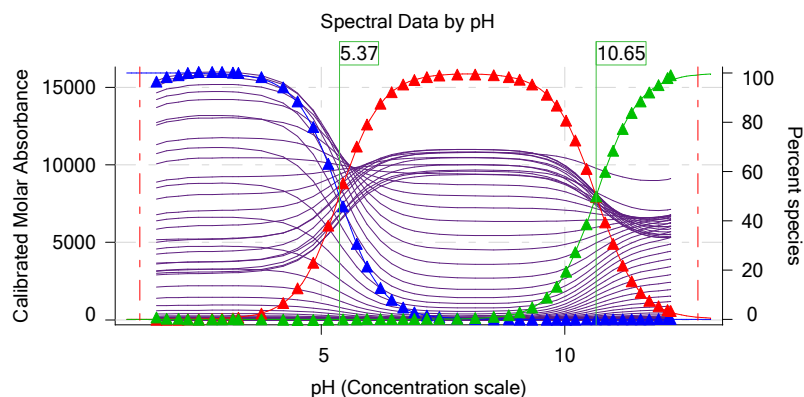
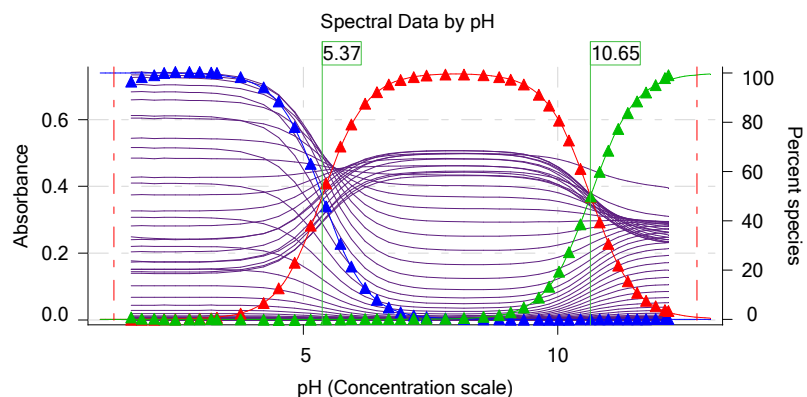
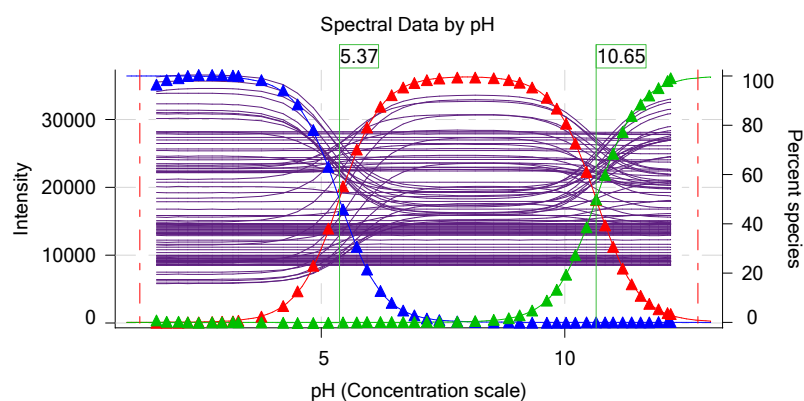
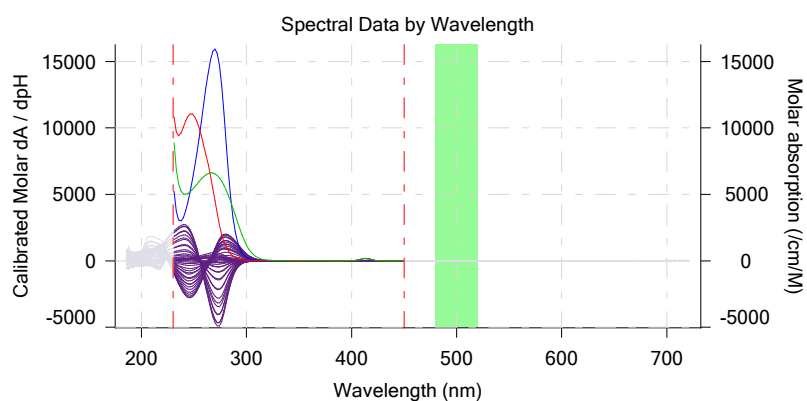
## Graphs



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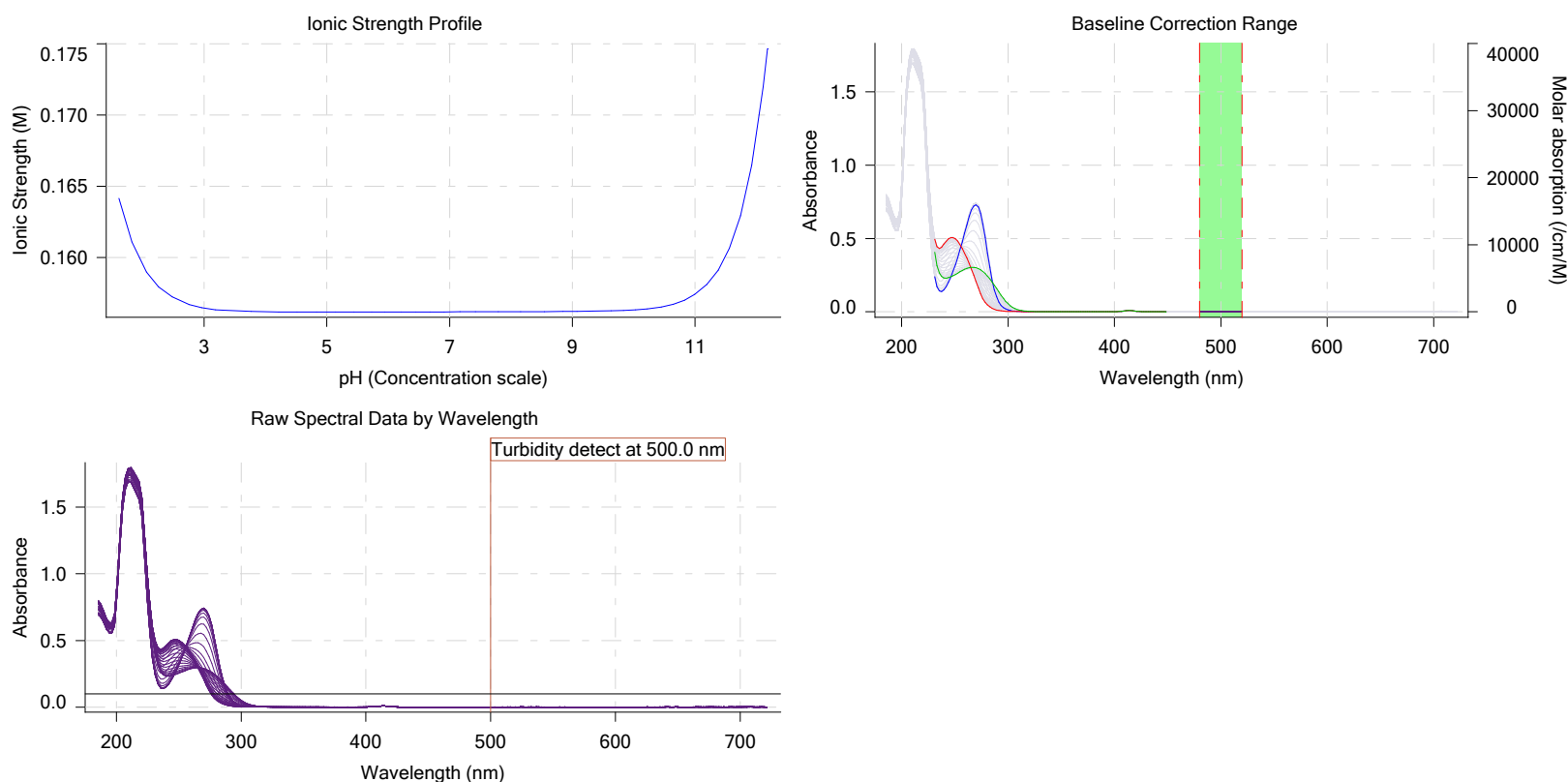
## Graphs (continued)



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## Graphs (continued)



## Assay Model

Settings	Value	Date/Time changed	Imported from
Sample name	M18	9/29/2017 5:35:09 PM	User entered value
Sample by	Volume		Default value
Sample volume	0.0010 mL	10/2/2017 2:08:50 PM	User entered value
Solvent	DMSO		Default value
Sample concentration	0.076700 M	10/2/2017 2:08:46 PM	User entered value
Solubility	Unknown		Default value
Molecular weight	267.11	9/29/2017 5:35:37 PM	User entered value
Individual pKa ionic environments	No		Default value
Number of pKas	2	9/29/2017 5:35:09 PM	User entered value
Sample is a	Ampholyte	9/29/2017 5:35:09 PM	User entered value
pKa 1	5.19	9/29/2017 5:35:09 PM	User entered value
Type	Base	9/29/2017 5:35:09 PM	User entered value
pKa 2	10.85	9/29/2017 5:35:09 PM	User entered value
Type	Acid	9/29/2017 5:35:09 PM	User entered value
logp (XH <sub>2</sub> +)	-10.00		Default value
logP (neutral XH)	-10.00	9/29/2017 5:35:09 PM	User entered value
logP (X -)	-10.00		Default value

## Events

Time	Event	Water	Acid	Base	Buffer	pH	dpH/dt	pH R-squared	pH SD
3:07.2	Dark spectrum								
3:08.6	Reference spectrum								
3:36.2	Volume reset due to vial change								
5:06.5	Initial pH = 7.65								
6:19.5	Data point 4	1.50000 mL	0.06910 mL	0.00000 mL	0.02500 mL	1.770	-0.01184	0.74426	0.00068
6:48.2	Data point 5	1.50000 mL	0.06910 mL	0.02519 mL	0.02500 mL	1.971	0.00746	0.48117	0.00053

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## Events (continued)

Time	Event	Water	Acid	Base	Buffer	pH	dpH/dt	pH R-squared	pH SD	dpH/dt time
7:05.2	Data point 6	1.50000 mL	0.06910 mL	0.04325 mL	0.02500 mL	2.207	0.00192	0.06751	0.00036	10.0 s
7:27.3	Data point 7	1.50000 mL	0.06910 mL	0.05235 mL	0.02500 mL	2.399	0.00661	0.57794	0.00043	10.0 s
7:44.0	Data point 8	1.50000 mL	0.06910 mL	0.05905 mL	0.02500 mL	2.619	0.01535	0.91692	0.00079	10.0 s
8:00.7	Data point 9	1.50000 mL	0.06910 mL	0.06303 mL	0.02500 mL	2.894	0.00716	0.72080	0.00042	10.0 s
8:32.6	Data point 10	1.50000 mL	0.06910 mL	0.06503 mL	0.02500 mL	3.096	0.00020	0.00157	0.00025	10.0 s
8:49.1	Data point 11	1.50000 mL	0.06910 mL	0.06637 mL	0.02500 mL	3.318	0.00835	0.78053	0.00047	10.0 s
9:05.6	Data point 12	1.50000 mL	0.06910 mL	0.06717 mL	0.02500 mL	3.429	0.00982	0.73586	0.00057	10.0 s
9:27.2	Data point 13	1.50000 mL	0.06910 mL	0.06837 mL	0.02500 mL	3.896	0.02565	0.97376	0.00128	10.0 s
9:48.8	Data point 14	1.50000 mL	0.06910 mL	0.06877 mL	0.02500 mL	4.343	0.04208	0.92645	0.00216	10.0 s
10:15.4	Data point 15	1.50000 mL	0.06910 mL	0.06903 mL	0.02500 mL	4.638	0.07366	0.94061	0.00375	10.0 s
10:36.9	Data point 16	1.50000 mL	0.06910 mL	0.06912 mL	0.02500 mL	4.953	0.09600	0.95575	0.00484	11.5 s
11:00.1	Data point 17	1.50000 mL	0.06910 mL	0.06919 mL	0.02500 mL	5.263	0.09324	0.96091	0.00470	13.5 s
11:25.1	Data point 18	1.50000 mL	0.06910 mL	0.06926 mL	0.02500 mL	5.563	0.08622	0.90727	0.00447	10.0 s
11:51.9	Data point 19	1.50000 mL	0.06910 mL	0.06933 mL	0.02500 mL	5.847	0.08887	0.86582	0.00472	10.0 s
12:13.4	Data point 20	1.50000 mL	0.06910 mL	0.06940 mL	0.02500 mL	6.057	0.06868	0.83099	0.00372	10.0 s
12:40.0	Data point 21	1.50000 mL	0.06910 mL	0.06950 mL	0.02500 mL	6.331	0.05483	0.79800	0.00303	10.0 s
13:06.6	Data point 22	1.50000 mL	0.06910 mL	0.06959 mL	0.02500 mL	6.560	0.05164	0.87560	0.00272	10.0 s
13:33.2	Data point 23	1.50000 mL	0.06910 mL	0.06968 mL	0.02500 mL	6.794	0.04797	0.85725	0.00256	10.0 s
14:05.0	Data point 24	1.50000 mL	0.06910 mL	0.06978 mL	0.02500 mL	7.022	0.06708	0.83114	0.00363	10.0 s
14:37.0	Data point 25	1.50000 mL	0.06910 mL	0.06987 mL	0.02500 mL	7.280	0.08699	0.86450	0.00462	10.5 s
15:04.3	Data point 26	1.50000 mL	0.06910 mL	0.06994 mL	0.02500 mL	7.532	0.08901	0.88489	0.00467	12.5 s
15:33.4	Data point 27	1.50000 mL	0.06910 mL	0.07001 mL	0.02500 mL	7.900	0.08966	0.82759	0.00487	14.5 s
15:59.5	Data point 28	1.50000 mL	0.06910 mL	0.07006 mL	0.02500 mL	8.262	0.07012	0.48968	0.00494	14.5 s
16:25.5	Data point 29	1.50000 mL	0.06910 mL	0.07011 mL	0.02500 mL	8.646	0.04130	0.31717	0.00362	13.0 s
16:50.0	Data point 30	1.50000 mL	0.06910 mL	0.07016 mL	0.02500 mL	8.952	0.08210	0.68201	0.00491	10.5 s
17:12.0	Data point 31	1.50000 mL	0.06910 mL	0.07020 mL	0.02500 mL	9.176	0.08618	0.77700	0.00483	10.0 s
17:33.7	Data point 32	1.50000 mL	0.06910 mL	0.07027 mL	0.02500 mL	9.430	0.06097	0.84648	0.00327	10.0 s
17:50.2	Data point 33	1.50000 mL	0.06910 mL	0.07037 mL	0.02500 mL	9.729	0.00948	0.22034	0.00100	10.0 s
18:11.7	Data point 34	1.50000 mL	0.06910 mL	0.07051 mL	0.02500 mL	9.929	-0.00526	0.29619	0.00048	10.0 s
18:38.5	Data point 35	1.50000 mL	0.06910 mL	0.07072 mL	0.02500 mL	10.121	-0.01816	0.87371	0.00096	10.0 s
19:05.0	Data point 36	1.50000 mL	0.06910 mL	0.07098 mL	0.02500 mL	10.314	-0.01844	0.96700	0.00093	10.0 s
19:31.8	Data point 37	1.50000 mL	0.06910 mL	0.07154 mL	0.02500 mL	10.538	-0.01466	0.85637	0.00078	10.0 s
19:48.4	Data point 38	1.50000 mL	0.06910 mL	0.07251 mL	0.02500 mL	10.737	-0.01498	0.90661	0.00078	10.0 s
20:05.0	Data point 39	1.50000 mL	0.06910 mL	0.07401 mL	0.02500 mL	10.916	-0.01520	0.90355	0.00079	10.0 s
20:21.7	Data point 40	1.50000 mL	0.06910 mL	0.07629 mL	0.02500 mL	11.080	-0.01402	0.87379	0.00074	10.0 s
20:48.4	Data point 41	1.50000 mL	0.06910 mL	0.07982 mL	0.02500 mL	11.277	-0.01765	0.95656	0.00089	10.0 s
21:05.1	Data point 42	1.50000 mL	0.06910 mL	0.08509 mL	0.02500 mL	11.461	-0.01097	0.79108	0.00061	10.0 s
21:22.0	Data point 43	1.50000 mL	0.06910 mL	0.09323 mL	0.02500 mL	11.642	-0.01171	0.88539	0.00061	10.0 s
21:38.8	Data point 44	1.50000 mL	0.06910 mL	0.10579 mL	0.02500 mL	11.817	-0.00740	0.74340	0.00042	10.0 s
21:55.8	Data point 45	1.50000 mL	0.06910 mL	0.12512 mL	0.02500 mL	11.996	-0.00623	0.63904	0.00038	10.0 s
22:13.2	Data point 46	1.50000 mL	0.06910 mL	0.15557 mL	0.02500 mL	12.175	-0.00249	0.18918	0.00028	10.0 s
22:30.2	Data point 47	1.50000 mL	0.06910 mL	0.17105 mL	0.02500 mL	12.239	-0.00549	0.55990	0.00036	10.0 s
24:35.7	Assay volumes	1.75000 mL	0.25475 mL	0.17538 mL	0.02500 mL					

## Assay Settings

Setting	Value	Original Value	Date/Time changed	Imported from
<b>General Settings</b>				
Analyst name	Dorothy Levorse			
Separate reference vial	Yes			
<b>Standard Experiment Settings</b>				
Number of titrations	1			
Minimum pH	1.800			
Maximum pH	12.200			
pH step between points of	0.200			
Minimum titrant addition	0.00002 mL			
Maximum titrant addition	0.10000 mL			
Argon flow rate	100%			

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## Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
Start titration using	Cautious pH adjust			
<b>Advanced General Settings</b>				
Detect turbidity using	Spectrometer			
Monitor at a wavelength of	500.0 nm			
Absorbance threshold of	0.100			
Collect turbidity sensor data	No			
Stir after titrant addition for	5 seconds			
For titrant addition, stir at	15%			
<b>Titration Pre-Dose</b>				
Titration pre-dose	None			
<b>Assay Medium</b>				
Cosolvent in use	No			
ISA water volume	1.50 mL			
Water added	Automatic			
After water addition, stir for	5 seconds			
At a speed of	15%			
Buffer in use	Yes			
Buffer type	Phosphate Buffer			
Volume of buffer introduced	0.025000 mL			
Add buffer manually	Manual			
After medium addition, stir for	5 seconds			
<b>Sample Sonication</b>				
Sonicate	No			
<b>Sample Dissolution</b>				
Perform a dissolution stage	No			
<b>Carbonate purge</b>				
Perform a carbonate purge	No			
<b>Temperature Control</b>				
Wait for temperature	Yes			
Required start temperature	25.0°C			
Acceptable deviation	0.5°C			
Time to wait	60 seconds			
Stir speed of	15%			
<b>Titration 1</b>				
Titrate from	Low to high pH			
Adjust to start pH	Yes			
After pH adjust stir for	10 seconds			
<b>Data Point Stability</b>				
Stir during data point collection	Yes			
For point collection, stir at	15%			
Delay before data point collection	0 seconds			
Number of points to average	20 points			
Time interval between points	0.50 seconds			
Required maximum standard deviation	0.00500 dpH/dt			
Stability timeout after	60 seconds			
<b>Experiment cleanup</b>				
Adjust pH to cleanup	To start pH			
And then stir for	60 seconds			
For cleaning, stir at	20%			
Then add water volume	0.25 mL			
And then stir for	30 seconds			

## Calibration Settings

Setting	Value	Date/Time changed	Imported from
Four-Plus alpha	0.150	10/3/2017 7:54:42 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Four-Plus S	0.9943	10/3/2017 7:54:42 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Four-Plus jH	0.6	10/3/2017 7:54:42 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r



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## Calibration Settings (continued)

Setting	Value	Date/Time changed	Imported from
Four-Plus jOH	-0.8	10/3/2017 7:54:42 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Base concentration factor	1.011	10/3/2017 7:54:42 PM	C:\Sirius_T3\KOH17I22.t3r
Acid concentration factor	1.007	10/3/2017 7:54:42 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r

## Instrument Settings

Setting	Value	Batch Id	Install date
Instrument owner	Merck		
Instrument ID	T311053		
Instrument type	T3 Simulator		
Software version	1.1.3.0		
Dispenser module		T3DM1100253	3/31/2009 5:24:52 AM
Dispenser 0	Water		3/31/2009 5:25:05 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Water (0.15 M KCl)	8-18-17	9/26/2017 8:05:04 AM
Dispenser 2	Acid		3/31/2009 5:25:11 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Acid (0.5 M HCl)	166940	9/8/2017 8:21:27 AM
Dispenser 1	Base		3/31/2009 5:25:21 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Base (0.5 M KOH)	9-22-17	9/22/2017 3:02:42 PM
Dispenser 5	Cosolvent		3/31/2009 5:26:24 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Distribution valve 5	Distribution Valve		3/31/2009 5:28:19 AM
Firmware version	1.1.3		
Port A	Methanol (80%, 0.15 M KCl)	9-26-17	9/29/2017 8:58:40 AM
Port B	Cyclohexane		9/19/2017 1:15:02 PM
Port C	MeCN (50%, 0.15 M KCl)	10-2-17	10/2/2017 10:28:55 AM
Dispenser 3	Buffer		8/3/2010 5:05:16 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Phosphate Buffer		9/12/2017 11:32:29 AM
Dispenser 6	Octanol		10/22/2010 10:52:43 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Octanol	9-14-17	9/14/2017 9:30:38 AM
Titration		T3TM1100153	3/31/2009 5:24:17 AM
Horizontal axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Probe I/O firmware version	1.1.1		
Electrode	T3 Electrode	T3E0769	8/15/2017 9:21:54 AM
E0 calibration	-9.53 mV		10/3/2017 7:55:06 PM
Filling solution	3M KCl	KCL095	10/2/2017 8:26:59 AM
Liquids			
Wash 1	50% IPA:50% Water		10/3/2017 8:05:00 AM
Wash 2	0.5% Triton X-100 in H2O		10/3/2017 8:05:01 AM
Buffer position 1	pH7 Wash		10/3/2017 8:05:03 AM
Buffer position 2	pH 7		10/3/2017 8:05:05 AM
Storage position			10/3/2017 8:05:10 AM
Wash water	8.3e+003 mL	10-3-17	10/3/2017 8:04:49 AM
Waste	1.7e+003 mL		10/3/2017 8:04:54 AM
Temperature controller			8/5/2010 6:35:13 AM
Turbidity detector			3/31/2009 5:24:45 AM



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## Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Spectrometer		072390	11/23/2010 11:22:28 AM
Dip probe		11086	
Wavelength coefficient A0	185.563		
Wavelength coefficient A1	2.17439		
Wavelength coefficient A2	-0.000285622		
Total lamp lit time	313:32:06		11/23/2010 11:22:28 AM
Calibrated on	9/26/2017 8:22:07 AM		
Integration time	11		
Scans averaged	10		
Autoloader		T3AL1100237	11/10/2015 9:34:13 AM
Left-right axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Front-back axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Vertical axis firmware version	1.17 AI1DI2DO2 Stepper 2		
Chassis I/O firmware version	1.11 AI1DI0DO4 Norgren I/O		
Configuration			
Alternate titration position	Titration position		
Alternate reference position	Reference position		
Maximum standard vial volume	3.50 mL		
Maximum alternate vial volume	25.00 mL		
Automatic action idle period	5 minute(s)		
Titrant tube volume	1.3 mL		
Syringe flush count	3.50		
Flowing wash pump volume	20.0 mL		
Flowing wash stir duration	5 s		
Flowing wash stir speed	30%		
Solvent wash stir duration	5 s		
Solvent wash stir speed	30%		
Surfactant wash stir duration	5 s		
Surfactant wash stir speed	30%		
E0 calibration minimum number of points	10		
E0 calibration maximum standard deviation	0.01500		
E0 calibration timeout period	60 s		
E0 calibration stir duration	5 s		
E0 calibration preparation stir speed	30%		
E0 calibration buffer wash stir duration	5 s		
E0 calibration buffer wash stir speed	30%		
E0 calibration reading stir speed	0%		
Spectrometer calibration stir duration	5 s		
Spectrometer calibration stir speed	30%		
Spectrometer calibration wash pump volume	20.0 mL		
Spectrometer calibration wash stir duration	5 s		
Spectrometer calibration wash stir speed	30%		
Overhead dispense height	10000		

## Refinement Settings

Setting	Value	Default value
Turbidity detection method	Spectrometer	Spectrometer
Turbidity wavelength to assess	500.0 nm	500.0 nm
Turbidity maximum absorbance	0.100	0.100
Turbidity probe threshold	50.00	50.00
Exclude turbid points	Yes	Yes
Low intensity warning threshold	100	100
Minimum absorbance change threshold	0.100	0.100
Eigenvector autocorrelation threshold	0.80	0.80
Maximum RMSD severe warning	0.250	0.250
Maximum RMSD warning	0.050	0.050