

Sample name: **M13**  
 Assay name: **UV-metric pKa**  
 Assay ID: **171-16013**  
 Filename: **C:\Sirius\_T3\Mehtap\20170915\_exp03\_uv\_M01-M14\171-16013\_M13\_UV-metric pKa.t3r**

Experiment start time: **9/16/2017 6:24:03 AM**  
 Analyst: **Dorothy Leverse**  
 Instrument ID: **T311053**

## Results

pKa 1 **5.76**  
 RMSD **0.003 0.002**  
 Chi squared **0.0112**  
 PCA calculated number of pKas **4**  
 Average ionic strength **0.158 M**  
 Average temperature **24.9°C**  
 Analyte concentration range **90.2 µM to 81.5 µM**

Number of pKas source **Manual (1)**  
 Wavelength clipping **230.0 nm to 450.0 nm**  
 pH clipping **1.282 to 12.706**

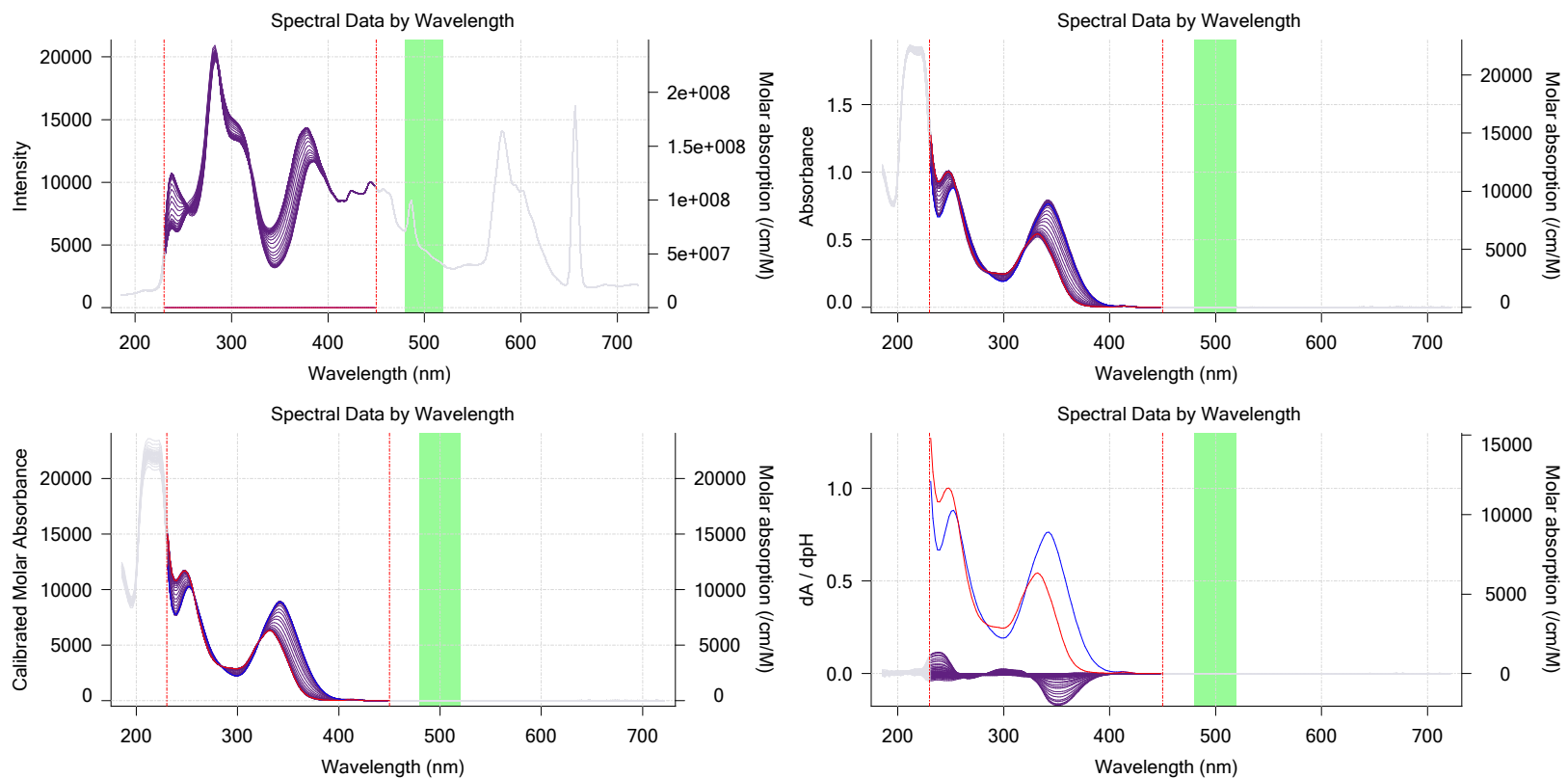
## Warnings and errors

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			

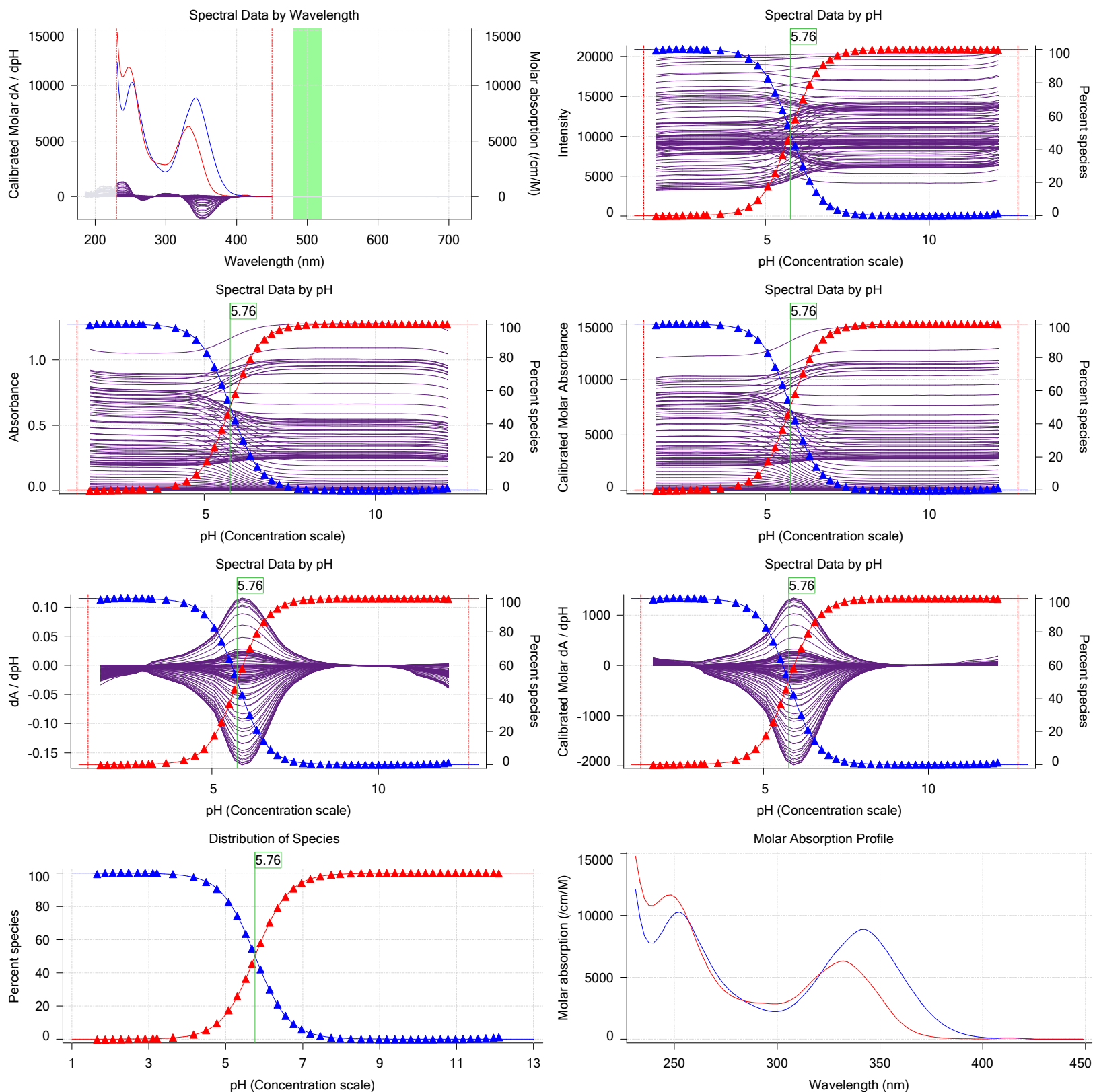
## Graphs



Sample name: **M13**  
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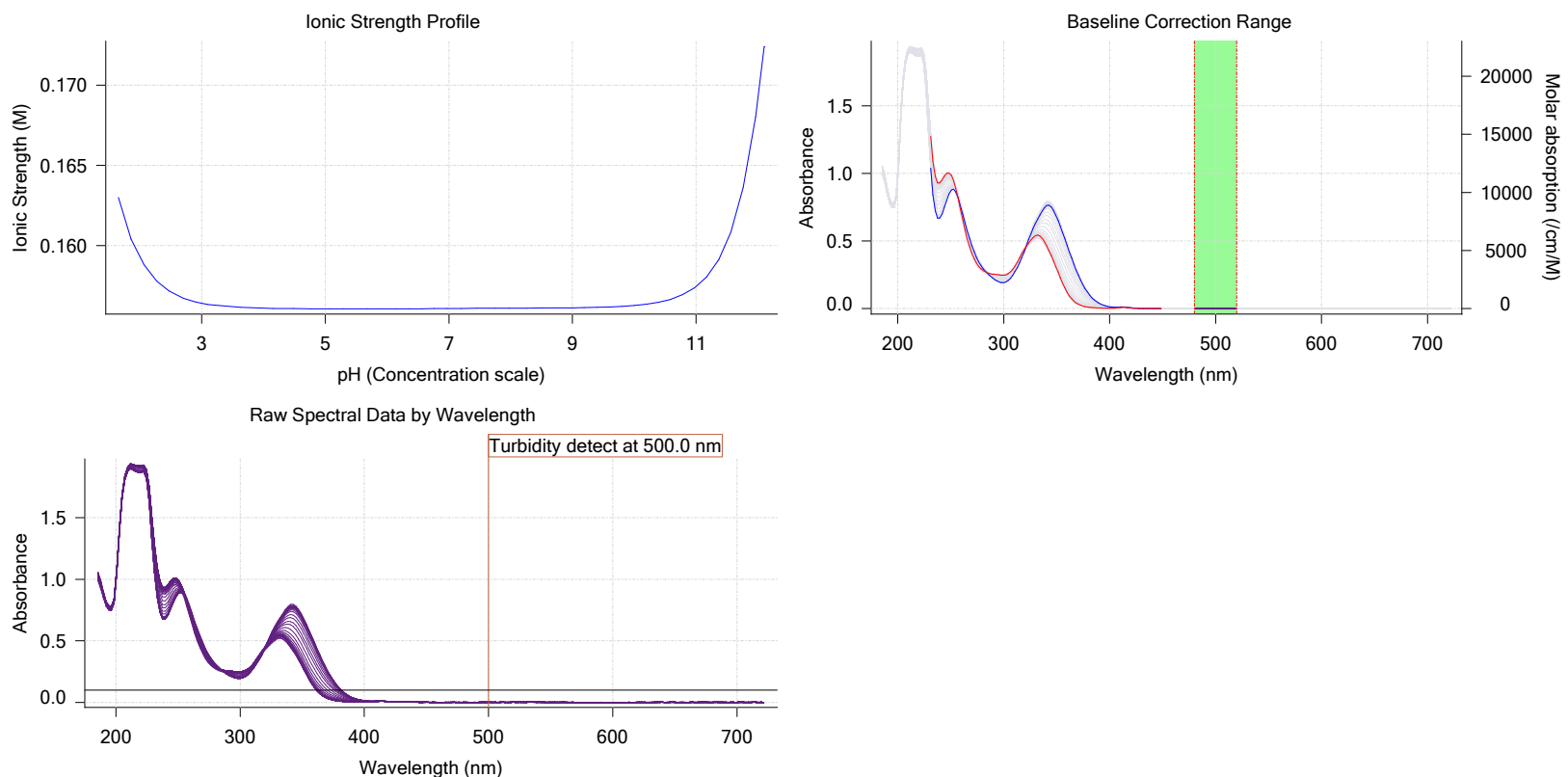
## Graphs (continued)



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## Graphs (continued)



## Events

Time	Event	Water	Acid	Base	Buffer	pH	dpH/dt	pH R-squared	pH SD
3:12.9	Dark spectrum								
3:14.3	Reference spectrum								
3:41.9	Volume reset due to vial change								
5:12.3	Initial pH = 7.57								
6:25.4	Data point 4	1.50000 mL	0.07107 mL	0.00000 mL	0.02500 mL	1.782	-0.00758	0.66308	0.0004
6:54.3	Data point 5	1.50000 mL	0.07107 mL	0.02521 mL	0.02500 mL	1.982	0.00508	0.20195	0.0005
7:11.3	Data point 6	1.50000 mL	0.07107 mL	0.04165 mL	0.02500 mL	2.183	-0.00220	0.08567	0.0003
7:28.2	Data point 7	1.50000 mL	0.07107 mL	0.05205 mL	0.02500 mL	2.390	0.01481	0.87532	0.0007
7:44.9	Data point 8	1.50000 mL	0.07107 mL	0.05844 mL	0.02500 mL	2.589	-0.00129	0.14410	0.0001
8:01.6	Data point 9	1.50000 mL	0.07107 mL	0.06254 mL	0.02500 mL	2.823	-0.00095	0.02911	0.0002
8:33.7	Data point 10	1.50000 mL	0.07107 mL	0.06489 mL	0.02500 mL	3.017	0.00185	0.16060	0.0002
8:50.3	Data point 11	1.50000 mL	0.07107 mL	0.06644 mL	0.02500 mL	3.216	-0.00008	0.00018	0.0002
9:06.8	Data point 12	1.50000 mL	0.07107 mL	0.06743 mL	0.02500 mL	3.324	0.00256	0.31007	0.0002
9:28.6	Data point 13	1.50000 mL	0.07107 mL	0.06884 mL	0.02500 mL	3.738	0.00844	0.82481	0.0004
9:50.3	Data point 14	1.50000 mL	0.07107 mL	0.06943 mL	0.02500 mL	4.275	0.02460	0.94246	0.0012
10:17.0	Data point 15	1.50000 mL	0.07107 mL	0.06971 mL	0.02500 mL	4.604	0.07931	0.93431	0.0040
10:38.6	Data point 16	1.50000 mL	0.07107 mL	0.06980 mL	0.02500 mL	4.881	0.09411	0.95848	0.0047
11:07.4	Data point 17	1.50000 mL	0.07107 mL	0.06987 mL	0.02500 mL	5.187	0.09931	0.97999	0.0049
11:31.5	Data point 18	1.50000 mL	0.07107 mL	0.06994 mL	0.02500 mL	5.407	0.08665	0.95517	0.0043
11:53.2	Data point 19	1.50000 mL	0.07107 mL	0.07001 mL	0.02500 mL	5.631	0.04830	0.88861	0.0025
12:09.7	Data point 20	1.50000 mL	0.07107 mL	0.07008 mL	0.02500 mL	5.797	0.03674	0.82425	0.0020
12:36.5	Data point 21	1.50000 mL	0.07107 mL	0.07018 mL	0.02500 mL	6.020	0.02639	0.79023	0.0014
13:03.2	Data point 22	1.50000 mL	0.07107 mL	0.07030 mL	0.02500 mL	6.256	0.00063	0.00236	0.0006
13:30.0	Data point 23	1.50000 mL	0.07107 mL	0.07041 mL	0.02500 mL	6.462	0.00792	0.29280	0.0007
14:01.9	Data point 24	1.50000 mL	0.07107 mL	0.07056 mL	0.02500 mL	6.668	0.02174	0.84333	0.0011
14:33.8	Data point 25	1.50000 mL	0.07107 mL	0.07072 mL	0.02500 mL	6.877	0.01170	0.49126	0.0008

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 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Events (continued)

Time	Event	Water	Acid	Base	Buffer	pH	dpH/dt	pH R-squared	pH SD	dpH/dt time
15:05.8	Data point 26	1.50000 mL	0.07107 mL	0.07086 mL	0.02500 mL	7.085	0.01955	0.75938	0.00111	10.0 s
15:42.6	Data point 27	1.50000 mL	0.07107 mL	0.07098 mL	0.02500 mL	7.312	0.04067	0.82375	0.00221	10.0 s
16:19.4	Data point 28	1.50000 mL	0.07107 mL	0.07110 mL	0.02500 mL	7.572	0.09224	0.90386	0.00479	10.0 s
16:51.0	Data point 29	1.50000 mL	0.07107 mL	0.07119 mL	0.02500 mL	7.858	0.08012	0.86079	0.00426	11.5 s
17:19.1	Data point 30	1.50000 mL	0.07107 mL	0.07126 mL	0.02500 mL	8.090	0.08092	0.80752	0.00444	13.0 s
17:48.8	Data point 31	1.50000 mL	0.07107 mL	0.07133 mL	0.02500 mL	8.370	0.08624	0.90623	0.00447	12.5 s
18:17.8	Data point 32	1.50000 mL	0.07107 mL	0.07140 mL	0.02500 mL	8.636	0.08020	0.77641	0.00449	10.5 s
18:45.2	Data point 33	1.50000 mL	0.07107 mL	0.07147 mL	0.02500 mL	8.868	0.07742	0.85091	0.00415	10.0 s
19:11.9	Data point 34	1.50000 mL	0.07107 mL	0.07157 mL	0.02500 mL	9.100	0.04967	0.85707	0.00265	10.0 s
19:38.4	Data point 35	1.50000 mL	0.07107 mL	0.07168 mL	0.02500 mL	9.307	0.01920	0.58942	0.00123	10.0 s
20:10.3	Data point 36	1.50000 mL	0.07107 mL	0.07185 mL	0.02500 mL	9.518	0.01959	0.80411	0.00108	10.0 s
20:37.2	Data point 37	1.50000 mL	0.07107 mL	0.07204 mL	0.02500 mL	9.727	0.00610	0.29008	0.00056	10.0 s
21:09.0	Data point 38	1.50000 mL	0.07107 mL	0.07230 mL	0.02500 mL	9.924	-0.00174	0.06407	0.00034	10.0 s
21:41.0	Data point 39	1.50000 mL	0.07107 mL	0.07265 mL	0.02500 mL	10.124	-0.00945	0.79796	0.00052	10.0 s
22:12.9	Data point 40	1.50000 mL	0.07107 mL	0.07314 mL	0.02500 mL	10.313	-0.01059	0.87851	0.00056	10.0 s
22:44.9	Data point 41	1.50000 mL	0.07107 mL	0.07387 mL	0.02500 mL	10.504	-0.01203	0.92998	0.00062	10.0 s
23:16.9	Data point 42	1.50000 mL	0.07107 mL	0.07502 mL	0.02500 mL	10.697	-0.01483	0.91051	0.00077	10.0 s
23:43.8	Data point 43	1.50000 mL	0.07107 mL	0.07702 mL	0.02500 mL	10.895	-0.01792	0.93671	0.00091	10.0 s
24:10.8	Data point 44	1.50000 mL	0.07107 mL	0.07937 mL	0.02500 mL	11.093	-0.01355	0.88481	0.00071	10.0 s
24:43.0	Data point 45	1.50000 mL	0.07107 mL	0.08302 mL	0.02500 mL	11.287	-0.01163	0.84856	0.00062	10.0 s
25:10.1	Data point 46	1.50000 mL	0.07107 mL	0.08958 mL	0.02500 mL	11.483	-0.00744	0.68520	0.00044	10.0 s
25:37.3	Data point 47	1.50000 mL	0.07107 mL	0.09965 mL	0.02500 mL	11.679	-0.00908	0.69666	0.00054	10.0 s
26:10.0	Data point 48	1.50000 mL	0.07107 mL	0.11670 mL	0.02500 mL	11.877	-0.00942	0.84252	0.00051	10.0 s
26:42.6	Data point 49	1.50000 mL	0.07107 mL	0.14353 mL	0.02500 mL	12.073	-0.00656	0.59806	0.00042	10.0 s
27:05.0	Data point 50	1.50000 mL	0.07107 mL	0.17159 mL	0.02500 mL	12.206	-0.00295	0.35582	0.00024	10.0 s
29:05.5	Assay volumes	1.75000 mL	0.25127 mL	0.17159 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%			
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>Titrant Pre-Dose</b>				
Titrant 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			

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## Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
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	0.112	9/16/2017 6:24:03 AM	C:\Sirius_T3\HCl17115.t3r
Four-Plus S	1.0006	9/16/2017 6:24:03 AM	C:\Sirius_T3\HCl17115.t3r
Four-Plus jH	0.7	9/16/2017 6:24:03 AM	C:\Sirius_T3\HCl17115.t3r
Four-Plus jOH	-0.6	9/16/2017 6:24:03 AM	C:\Sirius_T3\HCl17115.t3r
Base concentration factor	1.015	9/16/2017 6:24:03 AM	C:\Sirius_T3\KOH17111.t3r
Acid concentration factor	1.003	9/16/2017 6:24:03 AM	C:\Sirius_T3\HCl17115.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 6:24:52 AM
Dispenser 0	Water		3/31/2009 6:25:05 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Water (0.15 M KCl)	8-18-17	9/8/2017 9:22:43 AM
Dispenser 2	Acid		3/31/2009 6:25:11 AM



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**Instrument Settings (continued)**

Setting	Value	Batch Id	Install date
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Acid (0.5 M HCl)	166940	9/8/2017 9:21:27 AM
Dispenser 1	Base		3/31/2009 6:25:21 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Base (0.5 M KOH)	01/06/17	9/8/2017 9:20:03 AM
Dispenser 5	Cosolvent		3/31/2009 6:26:24 AM
Syringe volume	2.5 mL		
Firmware version	1.2.1(r2)		
Distribution valve 5	Distribution Valve		3/31/2009 6:28:19 AM
Firmware version	1.1.3		
Port A	Methanol (80%, 0.15 M KCl)	8-15-17	9/13/2017 12:23:11 PM
Dispenser 3	Buffer		8/3/2010 6:05:16 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Phosphate Buffer		9/12/2017 12:32:29 PM
Dispenser 6	Octanol		10/22/2010 11:52:43 AM
Syringe volume	0.5 mL		
Firmware version	1.2.1(r2)		
Titrant	Octanol	9-14-17	9/14/2017 10:30:38 AM
Titrator		T3TM1100153	3/31/2009 6:24:17 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 10:21:54 AM
E0 calibration	-9.10 mV		9/16/2017 6:24:27 AM
Filling solution	3M KCl	KCL095	9/13/2017 9:16:19 AM
Liquids			
Wash 1	50% IPA:50% Water		9/15/2017 9:38:18 AM
Wash 2	0.5% Triton X-100 in H2O		9/15/2017 9:38:22 AM
Buffer position 1	pH7 Wash		9/15/2017 9:38:24 AM
Buffer position 2	pH 7		9/15/2017 9:38:27 AM
Storage position			9/15/2017 9:38:55 AM
Wash water	2.9e+003 mL	9-11-17	9/11/2017 4:28:43 PM
Waste	7.2e+003 mL		9/11/2017 4:28:49 PM
Temperature controller			8/5/2010 7:35:13 AM
Turbidity detector			3/31/2009 6:24:45 AM
Spectrometer		072390	11/23/2010 12:22:28 PM
Dip probe		11086	
Wavelength coefficient A0	185.563		
Wavelength coefficient A1	2.17439		
Wavelength coefficient A2	-0.000285622		
Total lamp lit time	114:03:31		11/23/2010 12:22:28 PM
Calibrated on	9/6/2017 9:33:02 AM		
Integration time	11		
Scans averaged	10		
Autoloader		T3AL1100237	11/10/2015 10:34:13 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		





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**Instrument Settings (continued)**

Setting	Value	Batch Id	Install date
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