

Sample name: **M18**  
 Assay name: **UV-metric pKa**  
 Assay ID: **17J-03025**  
 Filename: **C:\Sirius\_T3\17J-03025\_M18\_UV-metric pKa.t3r**

Experiment start time: **10/3/2017 7:27:54 PM**  
 Analyst: **Dorothy Leverse**  
 Instrument ID: **T311053**

## Results

pKa 1 **5.37**  
 pKa 2 **10.64**  
 RMSD **0.006 0.003 0.003**  
 Chi squared **0.1536**  
 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 **1.276 to 12.734**

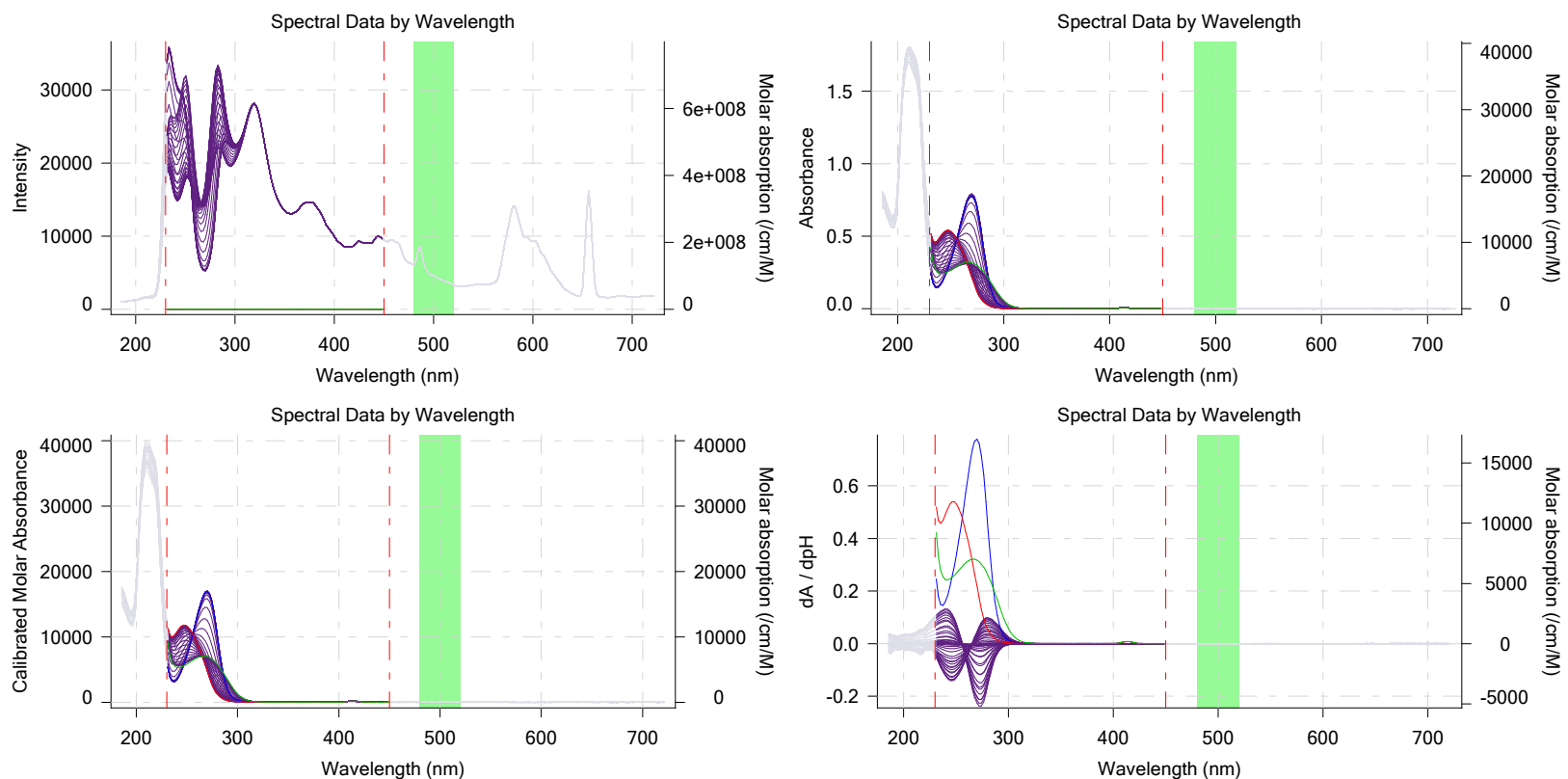
## Warnings and errors

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			

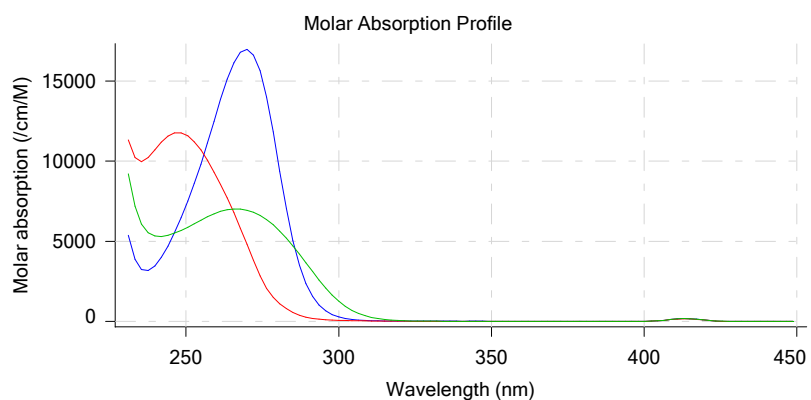
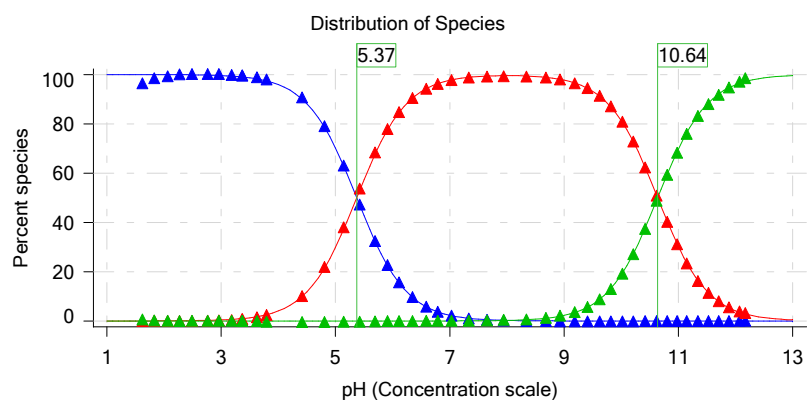
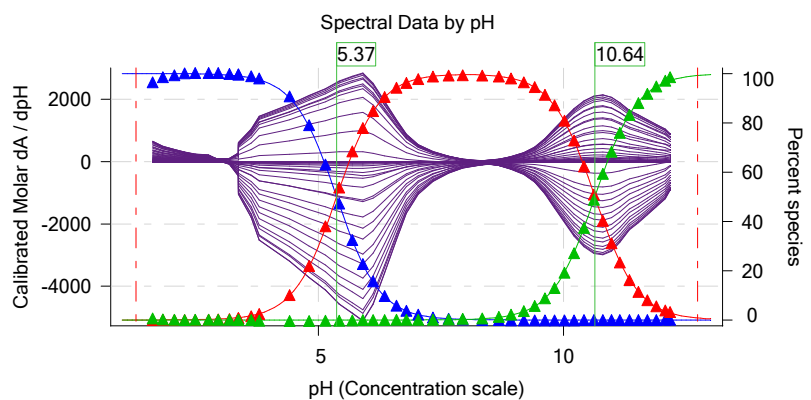
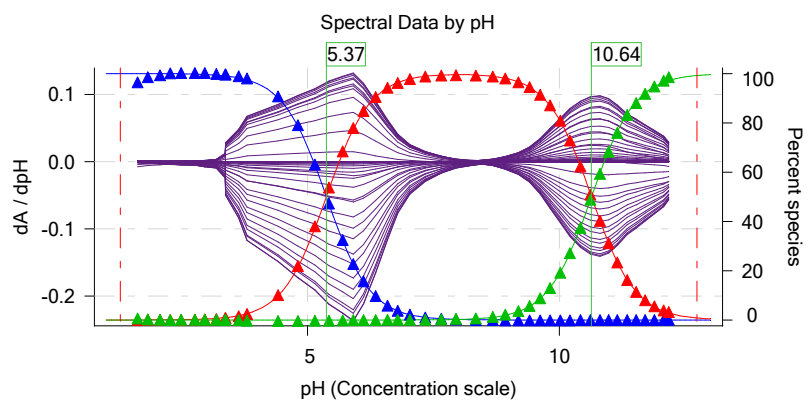
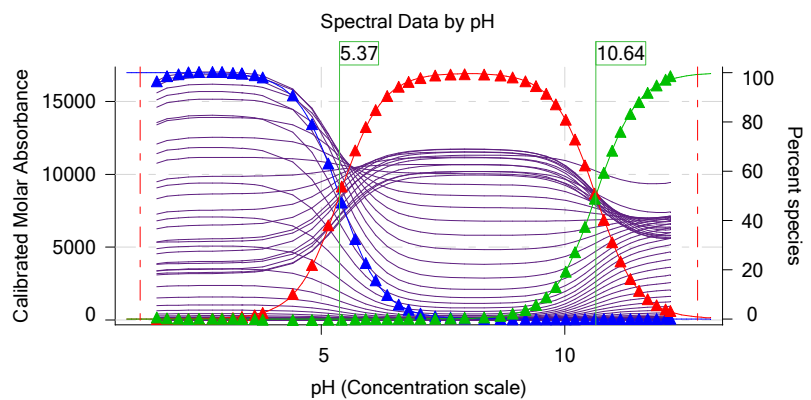
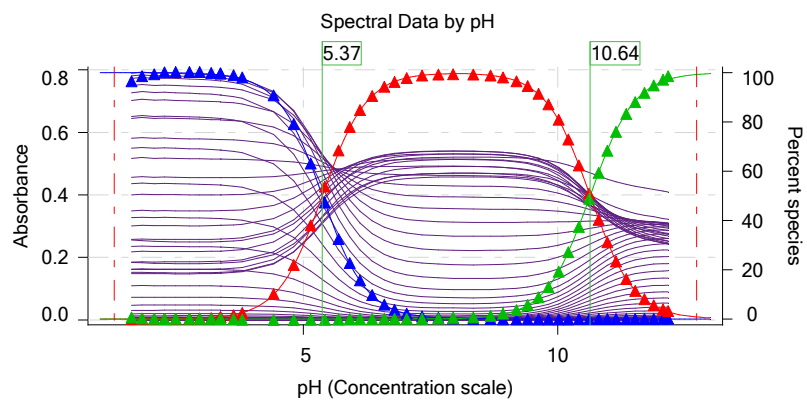
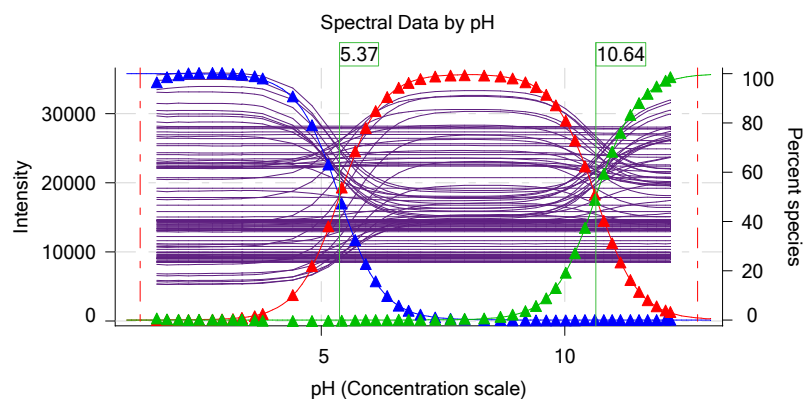
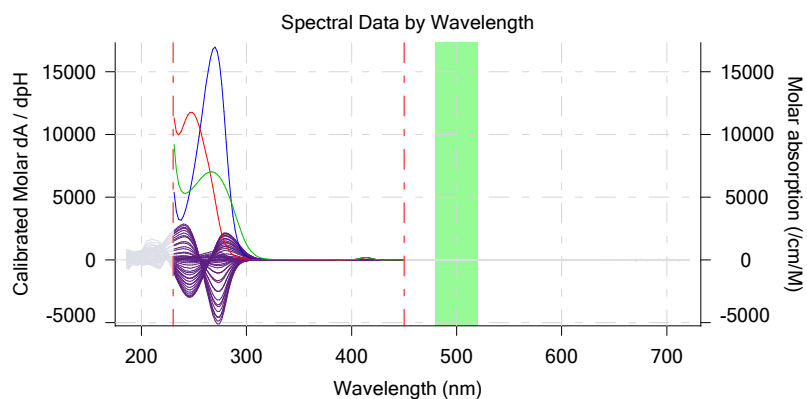
## Graphs



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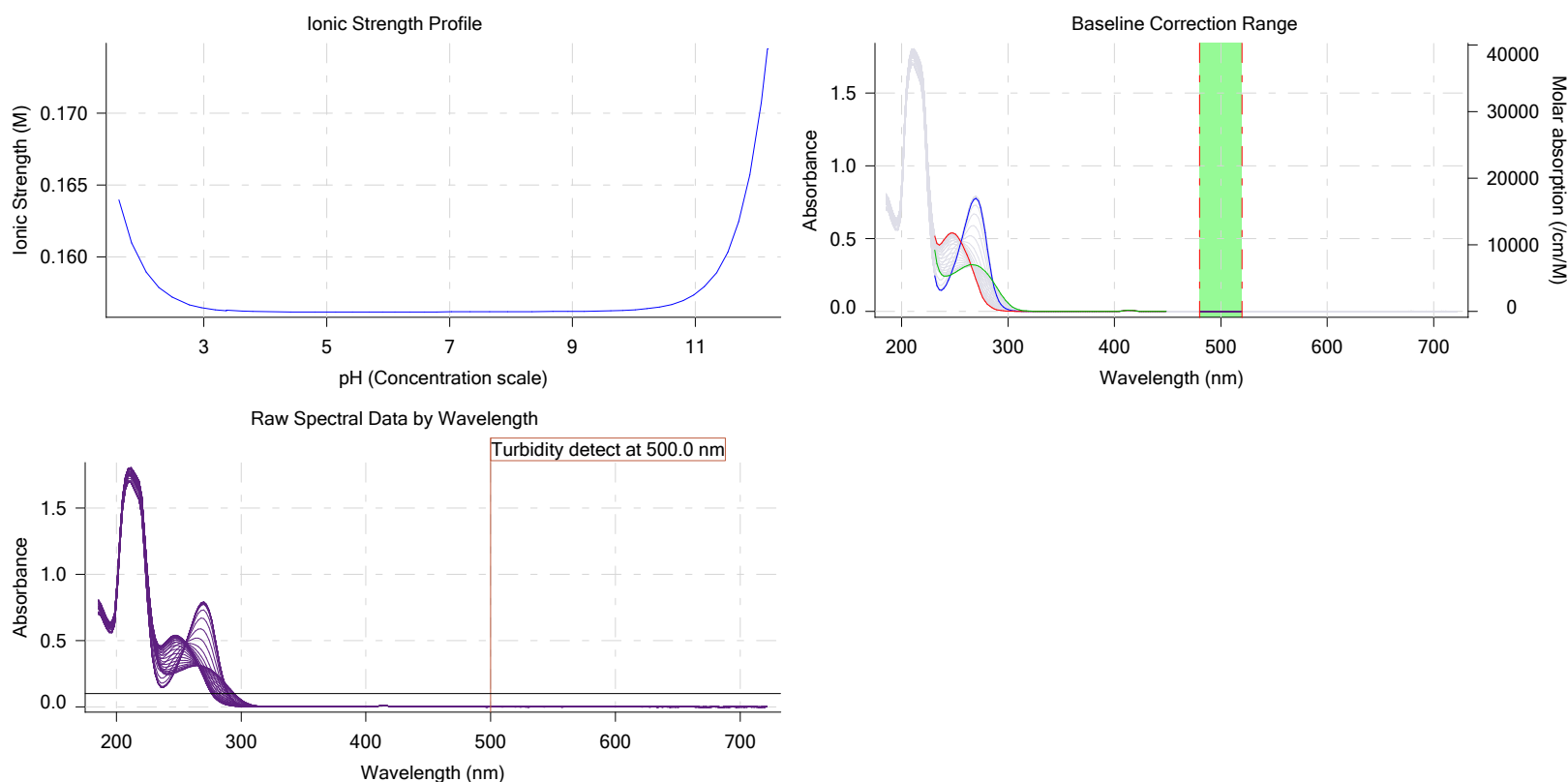
## Graphs (continued)



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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
3:22.8	Dark spectrum								
3:24.2	Reference spectrum								
3:51.8	Volume reset due to vial change								
5:22.1	Initial pH = 7.70								
6:35.0	Data point 4	1.50000 mL	0.06907 mL	0.00000 mL	0.02500 mL	1.776	-0.01051	0.77702	0.00059
7:03.6	Data point 5	1.50000 mL	0.06907 mL	0.02502 mL	0.02500 mL	1.977	0.00304	0.13184	0.00041

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**Events (continued)**

Time	Event	Water	Acid	Base	Buffer	pH	dpH/dt	pH R-squared	pH SD	dpH/dt time
7:20.6	Data point 6	1.50000 mL	0.06907 mL	0.04280 mL	0.02500 mL	2.208	-0.00034	0.00105	0.00052	10.0 s
7:47.4	Data point 7	1.50000 mL	0.06907 mL	0.05235 mL	0.02500 mL	2.410	-0.00313	0.25509	0.00031	10.0 s
8:04.2	Data point 8	1.50000 mL	0.06907 mL	0.05887 mL	0.02500 mL	2.625	0.01017	0.85679	0.00054	10.0 s
8:20.7	Data point 9	1.50000 mL	0.06907 mL	0.06282 mL	0.02500 mL	2.896	0.00731	0.86449	0.00039	10.0 s
8:52.5	Data point 10	1.50000 mL	0.06907 mL	0.06477 mL	0.02500 mL	3.093	0.00278	0.28457	0.00026	10.0 s
9:09.0	Data point 11	1.50000 mL	0.06907 mL	0.06611 mL	0.02500 mL	3.317	0.00574	0.69451	0.00034	10.0 s
9:25.6	Data point 12	1.50000 mL	0.06907 mL	0.06691 mL	0.02500 mL	3.498	0.00891	0.85036	0.00048	10.0 s
9:42.0	Data point 13	1.50000 mL	0.06907 mL	0.06743 mL	0.02500 mL	3.499	-0.00076	0.04078	0.00019	10.0 s
10:03.7	Data point 14	1.50000 mL	0.06907 mL	0.06802 mL	0.02500 mL	3.757	0.01586	0.91275	0.00082	10.0 s
10:20.2	Data point 15	1.50000 mL	0.06907 mL	0.06830 mL	0.02500 mL	3.923	0.02292	0.94755	0.00116	10.0 s
10:42.0	Data point 16	1.50000 mL	0.06907 mL	0.06867 mL	0.02500 mL	4.538	0.05620	0.95287	0.00284	10.0 s
11:08.5	Data point 17	1.50000 mL	0.06907 mL	0.06891 mL	0.02500 mL	4.930	0.07335	0.91370	0.00379	10.0 s
11:30.0	Data point 18	1.50000 mL	0.06907 mL	0.06900 mL	0.02500 mL	5.267	0.07568	0.96336	0.00380	10.0 s
11:51.6	Data point 19	1.50000 mL	0.06907 mL	0.06907 mL	0.02500 mL	5.541	0.07147	0.92129	0.00367	10.0 s
12:18.3	Data point 20	1.50000 mL	0.06907 mL	0.06914 mL	0.02500 mL	5.812	0.08568	0.93911	0.00436	10.5 s
12:40.2	Data point 21	1.50000 mL	0.06907 mL	0.06921 mL	0.02500 mL	6.026	0.06413	0.85160	0.00343	10.0 s
13:01.8	Data point 22	1.50000 mL	0.06907 mL	0.06928 mL	0.02500 mL	6.228	0.05804	0.85926	0.00309	10.0 s
13:28.3	Data point 23	1.50000 mL	0.06907 mL	0.06938 mL	0.02500 mL	6.464	0.04970	0.87659	0.00262	10.0 s
14:00.1	Data point 24	1.50000 mL	0.06907 mL	0.06950 mL	0.02500 mL	6.702	0.05547	0.87282	0.00293	10.0 s
14:26.8	Data point 25	1.50000 mL	0.06907 mL	0.06959 mL	0.02500 mL	6.904	0.06074	0.93483	0.00310	10.0 s
14:58.5	Data point 26	1.50000 mL	0.06907 mL	0.06968 mL	0.02500 mL	7.144	0.07530	0.88305	0.00396	10.0 s
15:30.0	Data point 27	1.50000 mL	0.06907 mL	0.06978 mL	0.02500 mL	7.445	0.08987	0.86909	0.00476	11.0 s
15:57.8	Data point 28	1.50000 mL	0.06907 mL	0.06985 mL	0.02500 mL	7.761	0.08322	0.83914	0.00448	13.0 s
16:22.5	Data point 29	1.50000 mL	0.06907 mL	0.06990 mL	0.02500 mL	8.051	0.07391	0.72960	0.00427	14.0 s
16:48.1	Data point 30	1.50000 mL	0.06907 mL	0.06994 mL	0.02500 mL	8.445	0.06998	0.63496	0.00434	14.0 s
17:13.6	Data point 31	1.50000 mL	0.06907 mL	0.06999 mL	0.02500 mL	8.783	0.07149	0.49787	0.00500	11.0 s
17:36.2	Data point 32	1.50000 mL	0.06907 mL	0.07004 mL	0.02500 mL	9.028	0.08207	0.80940	0.00450	10.5 s
17:58.2	Data point 33	1.50000 mL	0.06907 mL	0.07011 mL	0.02500 mL	9.288	0.07183	0.78421	0.00400	10.0 s
18:19.9	Data point 34	1.50000 mL	0.06907 mL	0.07018 mL	0.02500 mL	9.507	0.02095	0.54791	0.00140	10.0 s
18:41.6	Data point 35	1.50000 mL	0.06907 mL	0.07027 mL	0.02500 mL	9.718	0.01294	0.44968	0.00095	10.0 s
19:03.2	Data point 36	1.50000 mL	0.06907 mL	0.07039 mL	0.02500 mL	9.914	-0.00581	0.42213	0.00044	10.0 s
19:29.8	Data point 37	1.50000 mL	0.06907 mL	0.07058 mL	0.02500 mL	10.113	-0.01638	0.77045	0.00092	10.0 s
19:56.6	Data point 38	1.50000 mL	0.06907 mL	0.07088 mL	0.02500 mL	10.304	-0.01194	0.86135	0.00064	10.0 s
20:23.5	Data point 39	1.50000 mL	0.06907 mL	0.07135 mL	0.02500 mL	10.510	-0.01690	0.95309	0.00085	10.0 s
20:55.3	Data point 40	1.50000 mL	0.06907 mL	0.07230 mL	0.02500 mL	10.709	-0.01790	0.87987	0.00094	10.0 s
21:12.0	Data point 41	1.50000 mL	0.06907 mL	0.07371 mL	0.02500 mL	10.894	-0.01422	0.92935	0.00073	10.0 s
21:28.6	Data point 42	1.50000 mL	0.06907 mL	0.07587 mL	0.02500 mL	11.064	-0.01404	0.94566	0.00071	10.0 s
21:45.2	Data point 43	1.50000 mL	0.06907 mL	0.07907 mL	0.02500 mL	11.234	-0.01541	0.93019	0.00079	10.0 s
22:17.4	Data point 44	1.50000 mL	0.06907 mL	0.08403 mL	0.02500 mL	11.429	-0.01529	0.92357	0.00079	10.0 s
22:34.2	Data point 45	1.50000 mL	0.06907 mL	0.09158 mL	0.02500 mL	11.608	-0.01125	0.84686	0.00060	10.0 s
22:51.0	Data point 46	1.50000 mL	0.06907 mL	0.10315 mL	0.02500 mL	11.784	-0.01127	0.92404	0.00058	10.0 s
23:08.0	Data point 47	1.50000 mL	0.06907 mL	0.12102 mL	0.02500 mL	11.962	-0.00665	0.69098	0.00039	10.0 s
23:25.3	Data point 48	1.50000 mL	0.06907 mL	0.14896 mL	0.02500 mL	12.140	-0.00251	0.28015	0.00023	10.0 s
23:42.3	Data point 49	1.50000 mL	0.06907 mL	0.17067 mL	0.02500 mL	12.234	-0.00484	0.54727	0.00032	10.0 s
25:47.9	Assay volumes	1.75000 mL	0.25454 mL	0.17310 mL	0.02500 mL					

**Assay Settings**

Setting	Value	Original Value	Date/Time changed	Imported from
<b>General Settings</b>				
Analyst name	Dorothy Leverse			
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			

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

## Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
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			
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 7:27:54 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 S	0.9943	10/3/2017 7:27:54 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Four-Plus jH	0.6	10/3/2017 7:27:54 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Four-Plus jOH	-0.8	10/3/2017 7:27:54 PM	C:\Sirius_T3\17J-03018_Blank standardisation.t3r
Base concentration factor	1.011	10/3/2017 7:27:54 PM	C:\Sirius_T3\KOH17122.t3r
Acid concentration factor	1.007	10/3/2017 7:27:54 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
Titrator		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	-9.43 mV		10/3/2017 7:28:18 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	8.4e+003 mL	10-3-17	10/3/2017 8:04:49 AM
Waste	1.6e+003 mL		10/3/2017 8:04:54 AM



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## Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Temperature controller			8/5/2010 6:35:13 AM
Turbidity detector			3/31/2009 5:24:45 AM
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



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Assay name:	<b>UV-metric pKa</b>	Analyst:	<b>Dorothy Levorse</b>
Assay ID:	<b>17J-03025</b>	Instrument ID:	<b>T311053</b>
Filename:	<b>C:\Sirius_T3\17J-03025_M18_UV-metric pKa.t3r</b>		

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### Refinement Settings (continued)

Setting	Value	Default value
Maximum RMSD warning	0.050	0.050

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