

Sample name: **M06**  
Assay name: **UV-metric psKa**  
Assay ID: **17K-16011**  
Filename: **C:\Sirius\_T3\Mehtap\20171116\_exp18\_pKa\17K-16011\_M06\_UV-metric psKa.t3r**

Experiment start time: **11/16/2017 12:23:06 PM**  
Analyst: **Dorothy Levorse**  
Instrument ID: **T311053**

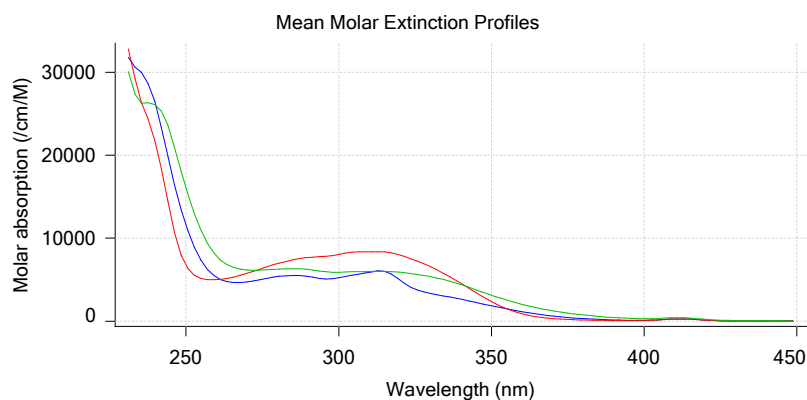
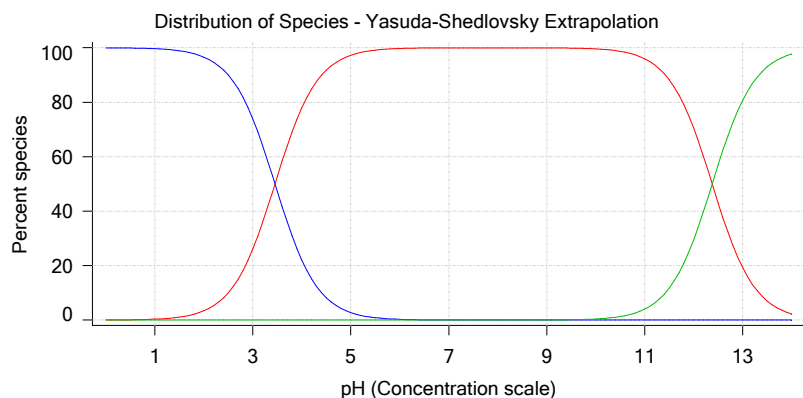
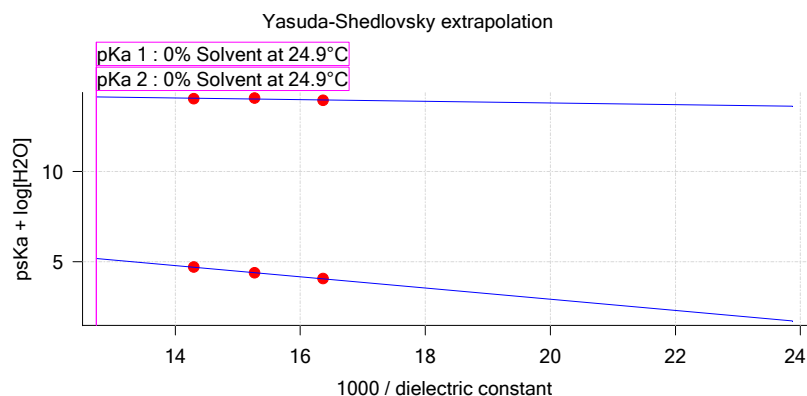
## Yasuda-Shedlovsky result

Extrapolation type	pKa 0%	SD	Intercept	Slope	R <sup>2</sup>	Ionic strength	Temperature
Yasuda-Shedlovsky	3.45	±0.02	9.17	-311.9039	0.9994	0.163 M	24.9°C
Yasuda-Shedlovsky	12.38	±0.14	14.69	-44.9279	0.4440	0.163 M	24.9°C

## Component assay results

Titration	Methanol weight%	Direction	Result type	Dielectric constant	[H2O]	Ionic strength	Temperature	psKa 1	psKa 2
17K-16011 Points 4 to 41	39.80 %	Up	UV-metric pKa	61.1	30.1 M	0.156 M	24.9°C	✓	12.45
17K-16011 Points 43 to 84	30.12 %	Up	UV-metric pKa	65.5	35.8 M	0.164 M	24.9°C	✓	12.51
17K-16011 Points 86 to 130	20.05 %	Up	UV-metric pKa	69.9	42.0 M	0.169 M	24.9°C	✓	12.40

## Graphs



## UV-metric psKa Titration 1 of 3 17K-16011 Points 4 to 41

### Results

pKa 1 **2.59**  
pKa 2 **12.45**  
RMSD **0.002 0.002 0.002**  
Chi squared **0.0676**  
PCA calculated number of pKas **2**  
Average ionic strength **0.156 M**  
Average temperature **24.9°C**  
Analyte concentration range **43.1 µM to 40.8 µM**  
Methanol weight % **39.8 %**  
Dielectric constant **61.1**  
Water concentration **30.1 M**

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Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Results (continued)

Number of pKas source **Predicted**  
 Wavelength clipping **236.0 nm to 450.0 nm**  
 pH clipping **1.463 to 12.541**

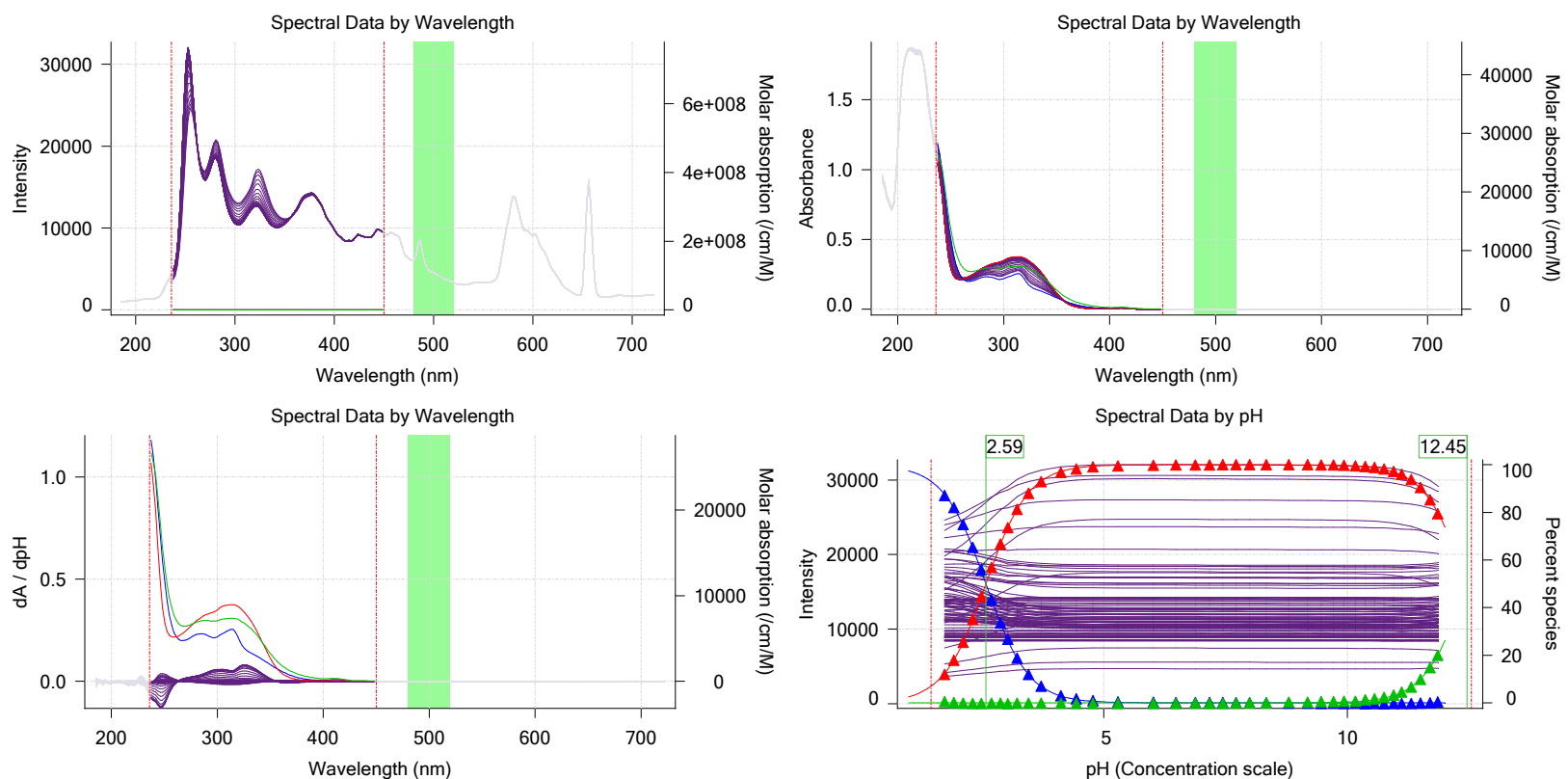
## Warnings and errors

Errors None  
 Warnings None

## 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			

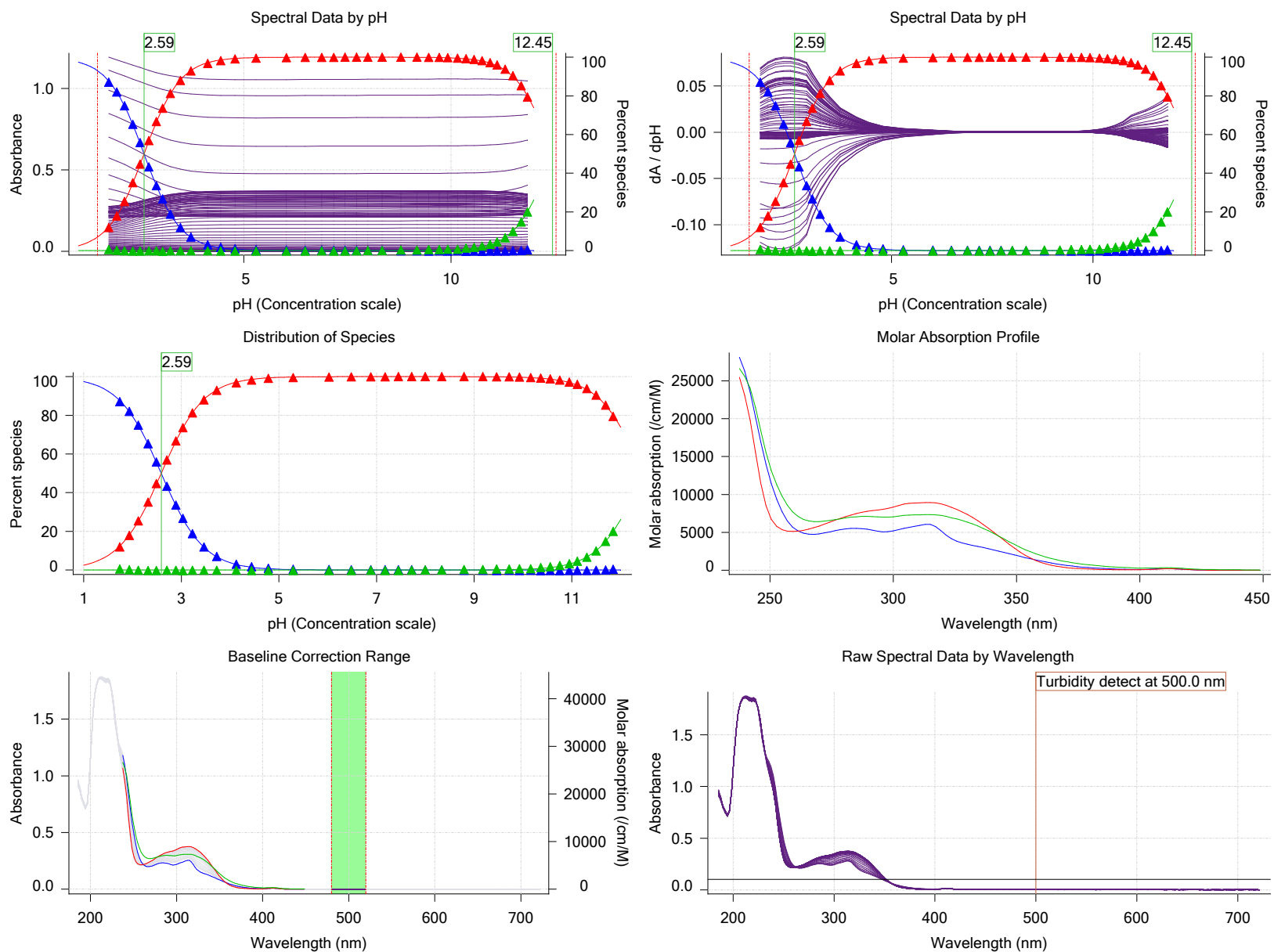
## Graphs



Sample name: **M06**  
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Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Leverse**  
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## Graphs (continued)



UV-metric psKa Titration 2 of 3 17K-16011 Points 43 to 84

## Results

pKa 1 **2.84**  
 pKa 2 **12.51**  
 RMSD **0.001 0.001 0.001**  
 Chi squared **0.0187**  
 PCA calculated number of pKas **3**  
 Average ionic strength **0.164 M**  
 Average temperature **24.9°C**  
 Analyte concentration range **33.3 µM to 31.5 µM**  
 Methanol weight % **30.1 %**  
 Dielectric constant **65.5**  
 Water concentration **35.8 M**

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Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Results (continued)

Number of pKas source **Predicted**  
 Wavelength clipping **230.0 nm to 450.0 nm**  
 pH clipping **1.478 to 12.549**

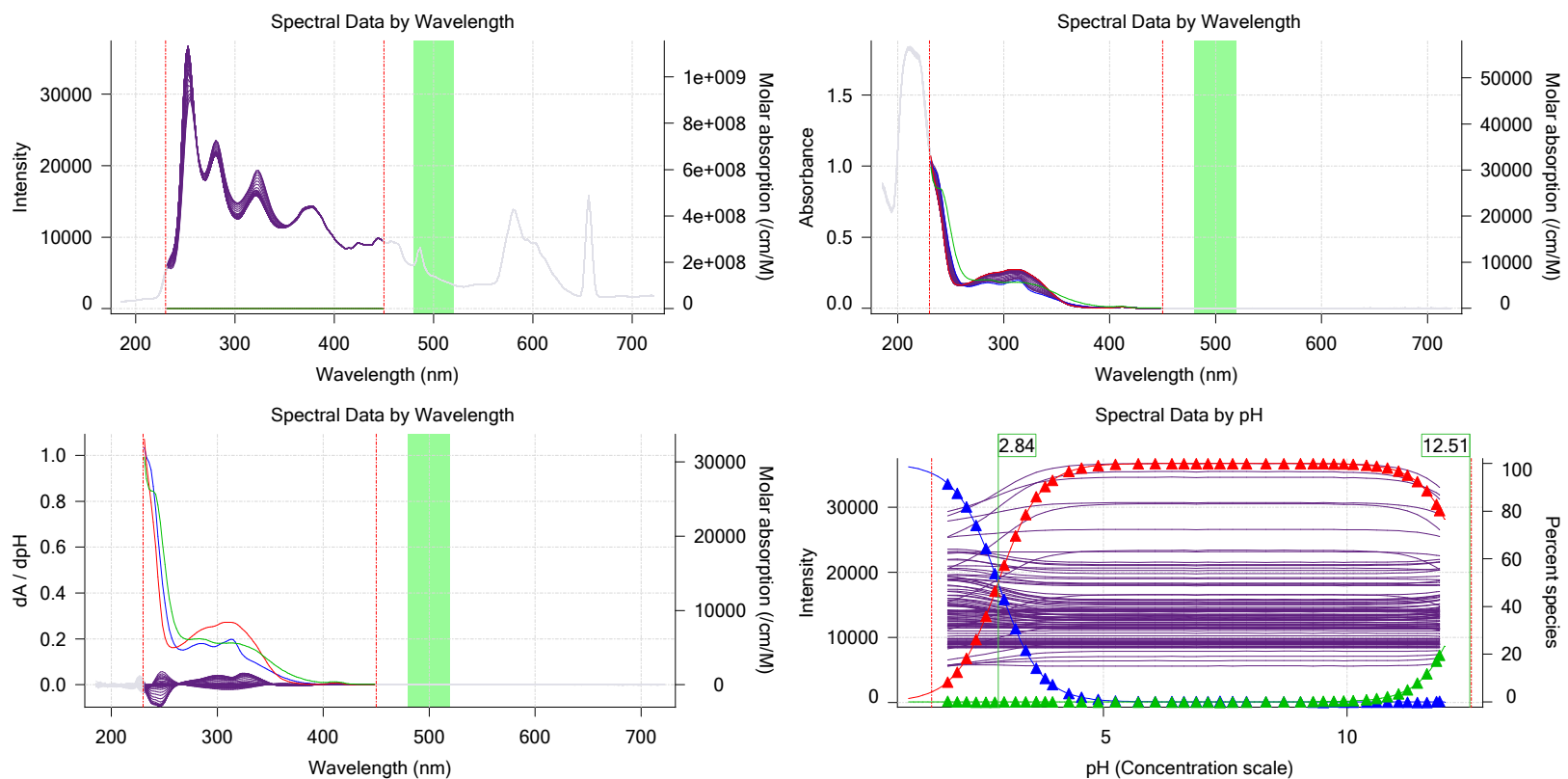
## Warnings and errors

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			

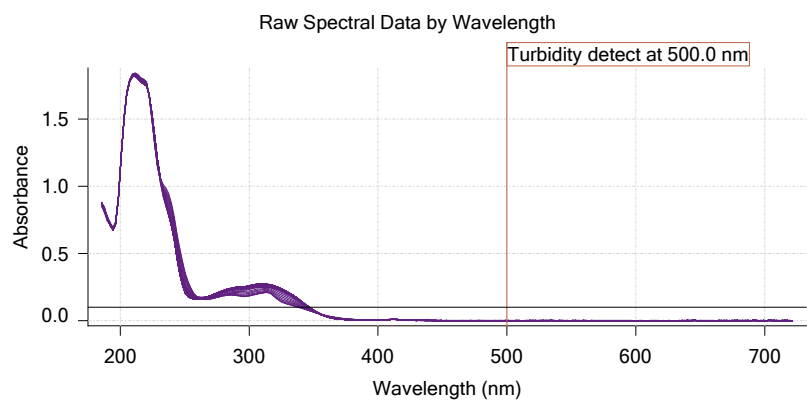
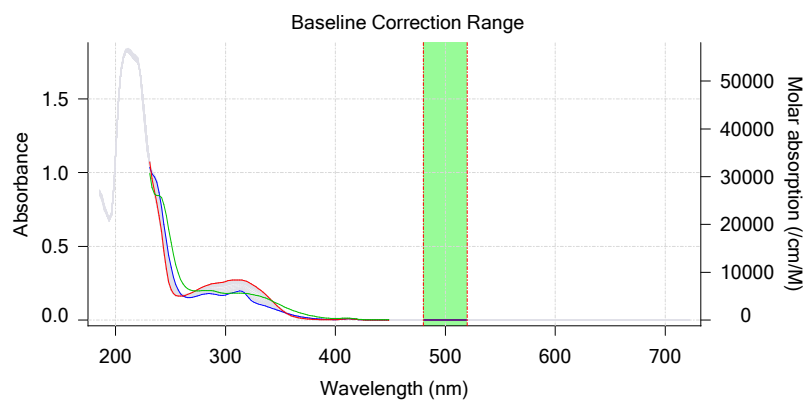
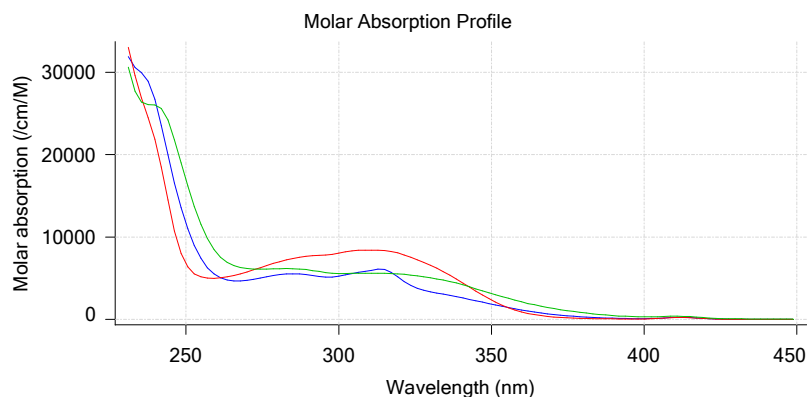
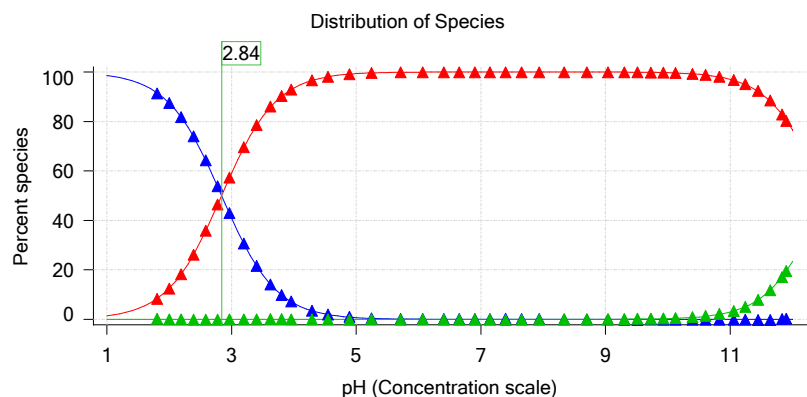
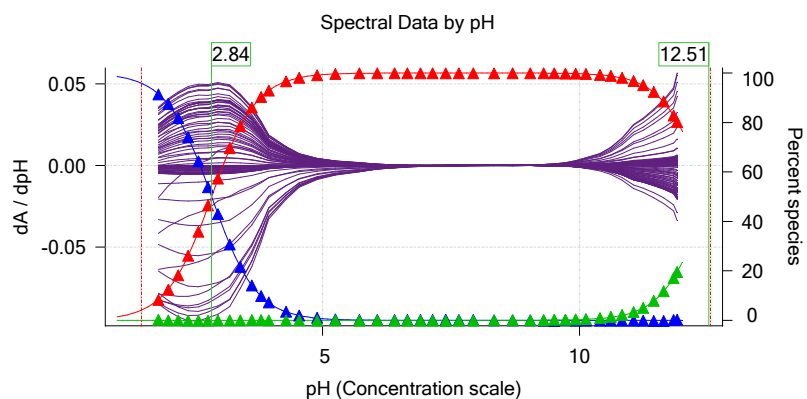
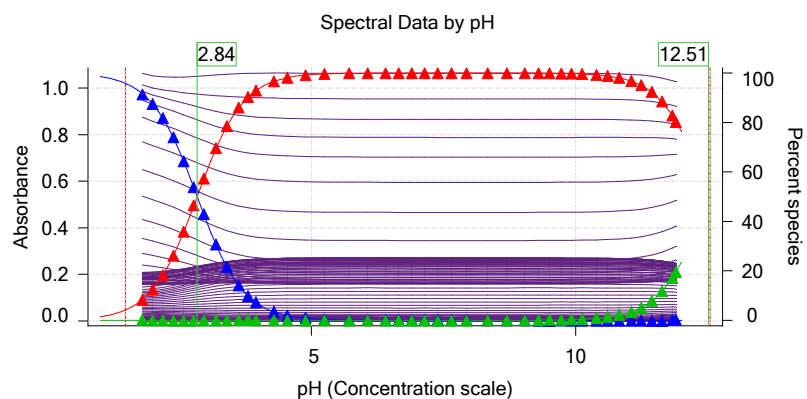
## Graphs



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Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Graphs (continued)



UV-metric psKa Titration 3 of 3 17K-16011 Points 86 to 130

## Results

pKa 1 **3.09**  
 pKa 2 **12.40**  
 RMSD **0.005 0.003 0.004**  
 Chi squared **0.1244**  
 PCA calculated number of pKas **2**  
 Average ionic strength **0.169 M**  
 Average temperature **24.9°C**  
 Analyte concentration range **22.6 µM to 21.5 µM**  
 Methanol weight % **20.1 %**  
 Dielectric constant **69.9**  
 Water concentration **42.0 M**

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 Analyst: **Dorothy Levorse**  
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## Results (continued)

Number of pKas source **Predicted**  
 Wavelength clipping **230.0 nm to 450.0 nm**  
 pH clipping **1.491 to 12.560**

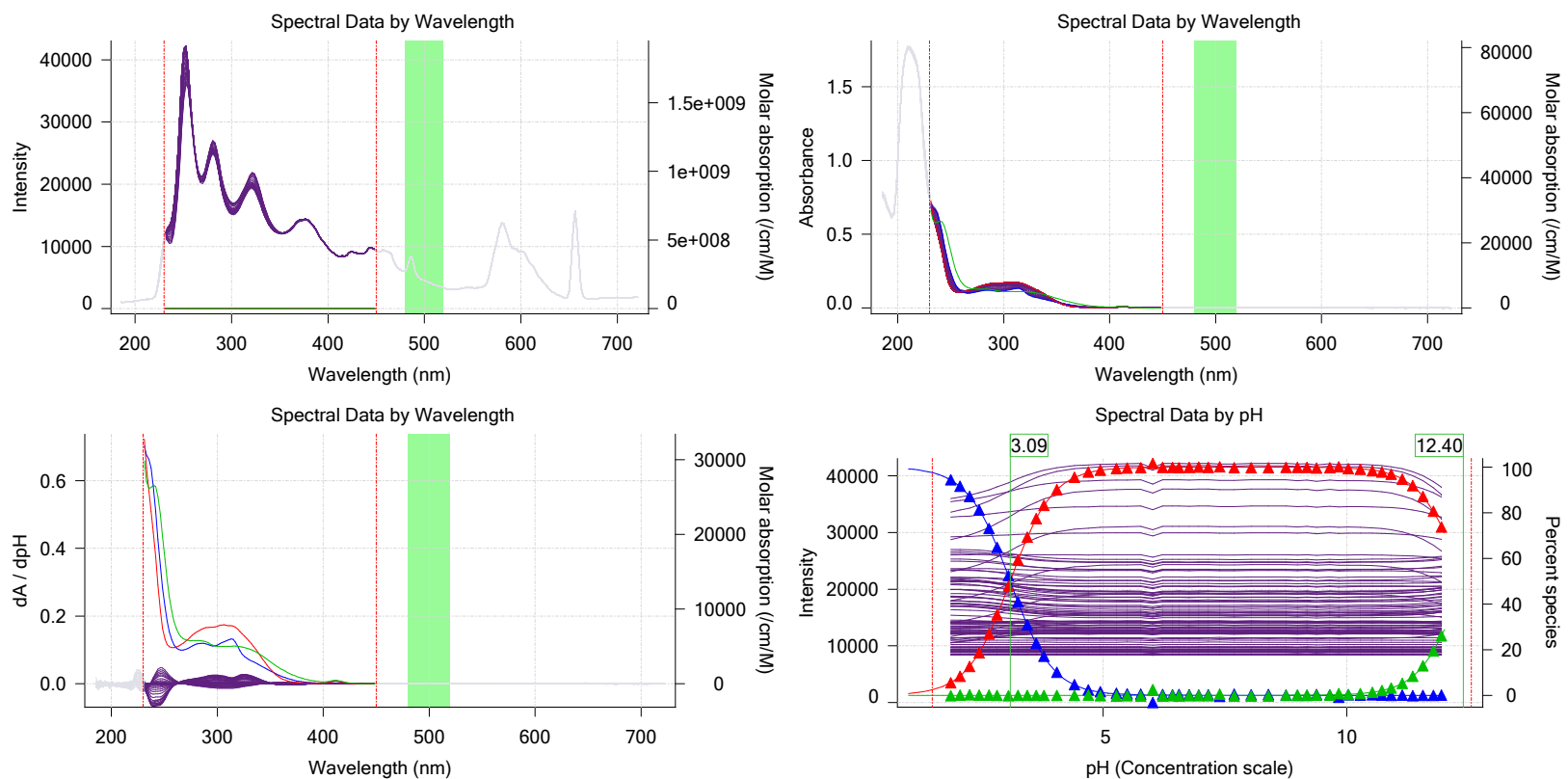
## Warnings and errors

Errors None  
 Warnings None

## 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			

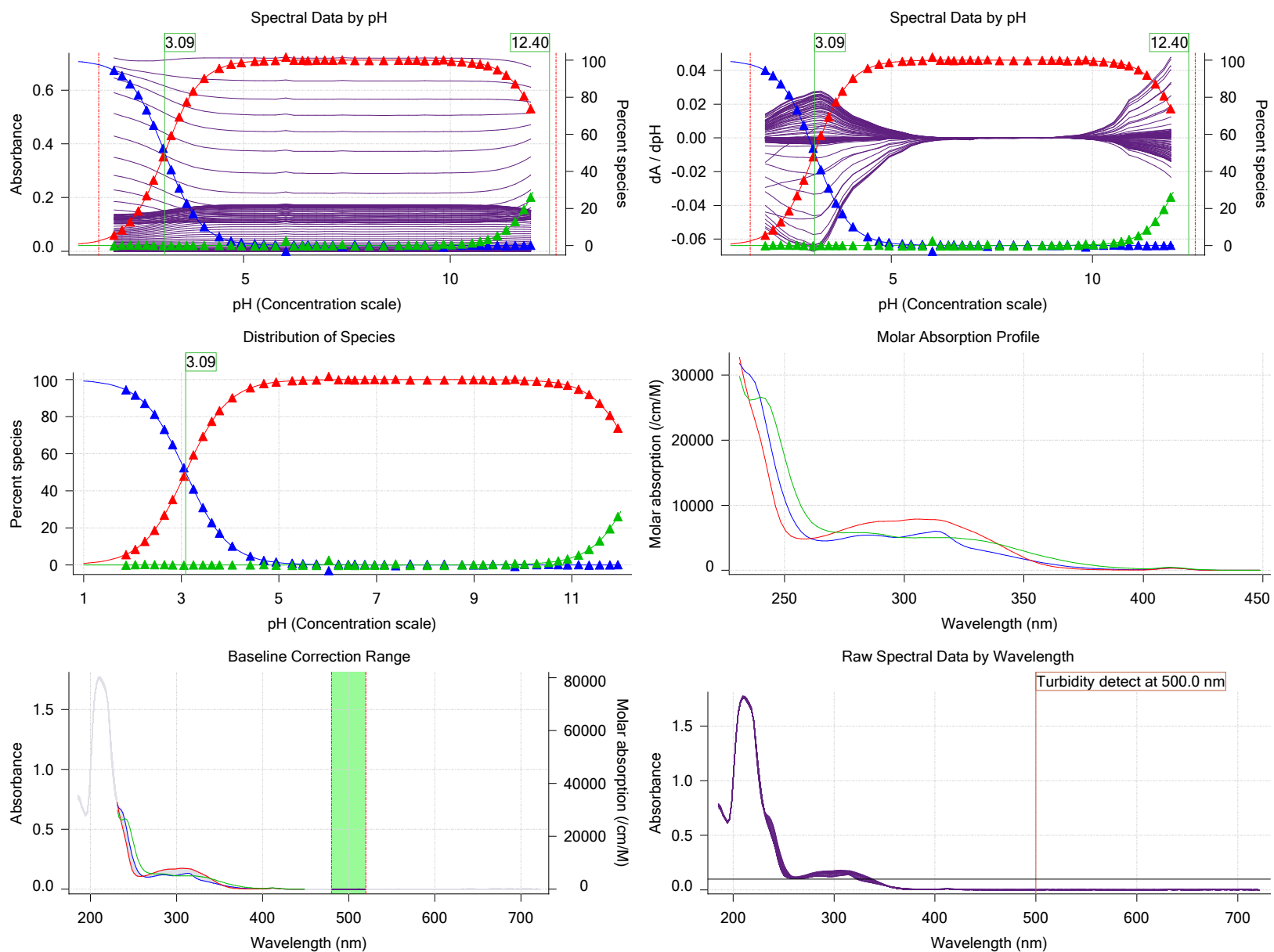
## Graphs



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Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Graphs (continued)



## Assay Model

### Settings

Settings	Value	Date/Time changed	Imported from
Sample name	M06	11/9/2017 5:26:58 PM	User entered value
Sample by	Volume		Default value
Sample volume	0.0020 mL	11/16/2017 11:08:41 AM	User entered value
Solvent	DMSO		Default value
Sample concentration	0.034300 M	11/16/2017 11:09:11 AM	User entered value
Solubility	Unknown		Default value
Molecular weight	328.16	11/9/2017 5:27:07 PM	User entered value
Individual pKa ionic environments	No		Default value
Number of pKas	2	11/9/2017 5:26:58 PM	User entered value
Sample is a	Ampholyte	11/9/2017 5:26:58 PM	User entered value
pKa 1	3.03	11/9/2017 5:26:58 PM	User entered value
Type	Base	11/9/2017 5:26:58 PM	User entered value
pKa 2	11.74	11/9/2017 5:26:58 PM	User entered value
Type	Acid	11/9/2017 5:26:58 PM	User entered value



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 Instrument ID: **T311053**

## Assay Model (continued)

Settings	Value	Date/Time changed	Imported from
logp (XH2 +)	-10.00		Default value
logP (neutral XH)	-10.00	11/9/2017 5:26:58 PM	User entered value
logP (X -)	-10.00		Default value

## Events

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared
3:33.5	Dark spectrum								
3:34.9	Reference spectrum								
4:02.5	Volume reset due to vial change								
4:46.7	Initial pH = 8.20								
5:59.5	Data point 4	0.55997 mL	0.06286 mL	0.00000 mL	0.94003 mL	0.02500 mL	1.963	-0.01812	0.85617
6:28.1	Data point 5	0.55997 mL	0.06286 mL	0.02274 mL	0.94003 mL	0.02500 mL	2.159	-0.00520	0.13999
6:45.0	Data point 6	0.55997 mL	0.06286 mL	0.03624 mL	0.94003 mL	0.02500 mL	2.346	0.02483	0.59291
7:01.8	Data point 7	0.55997 mL	0.06286 mL	0.04525 mL	0.94003 mL	0.02500 mL	2.547	0.00707	0.64665
7:18.6	Data point 8	0.55997 mL	0.06286 mL	0.05101 mL	0.94003 mL	0.02500 mL	2.721	0.01012	0.84960
7:35.4	Data point 9	0.55997 mL	0.06286 mL	0.05489 mL	0.94003 mL	0.02500 mL	2.935	0.01490	0.92708
7:52.1	Data point 10	0.55997 mL	0.06286 mL	0.05727 mL	0.94003 mL	0.02500 mL	3.118	0.01507	0.89399
8:08.6	Data point 11	0.55997 mL	0.06286 mL	0.05884 mL	0.94003 mL	0.02500 mL	3.264	0.01020	0.83871
8:30.2	Data point 12	0.55997 mL	0.06286 mL	0.05992 mL	0.94003 mL	0.02500 mL	3.456	0.01261	0.84340
8:46.7	Data point 13	0.55997 mL	0.06286 mL	0.06065 mL	0.94003 mL	0.02500 mL	3.691	0.04066	0.94019
9:08.3	Data point 14	0.55997 mL	0.06286 mL	0.06119 mL	0.94003 mL	0.02500 mL	3.950	0.05959	0.98706
9:30.0	Data point 15	0.55997 mL	0.06286 mL	0.06162 mL	0.94003 mL	0.02500 mL	4.349	0.10041	0.98571
9:54.4	Data point 16	0.55997 mL	0.06286 mL	0.06181 mL	0.94003 mL	0.02500 mL	4.663	0.09777	0.98645
10:37.0	Data point 17	0.55997 mL	0.06286 mL	0.06192 mL	0.94003 mL	0.02500 mL	5.007	0.09836	0.98667
11:32.6	Data point 18	0.55997 mL	0.06286 mL	0.06199 mL	0.94003 mL	0.02500 mL	5.512	0.09667	0.95254
12:35.7	Data point 19	0.55997 mL	0.06286 mL	0.06206 mL	0.94003 mL	0.02500 mL	6.241	0.09630	0.96924
13:34.0	Data point 20	0.55997 mL	0.06286 mL	0.06214 mL	0.94003 mL	0.02500 mL	6.698	0.09317	0.96359
14:20.8	Data point 21	0.55997 mL	0.06286 mL	0.06223 mL	0.94003 mL	0.02500 mL	7.095	0.09780	0.98112
15:06.0	Data point 22	0.55997 mL	0.06286 mL	0.06235 mL	0.94003 mL	0.02500 mL	7.385	0.09780	0.98614
15:47.2	Data point 23	0.55997 mL	0.06286 mL	0.06246 mL	0.94003 mL	0.02500 mL	7.656	0.10026	0.98935
16:33.5	Data point 24	0.55997 mL	0.06286 mL	0.06258 mL	0.94003 mL	0.02500 mL	7.928	0.09973	0.97805
17:13.6	Data point 25	0.55997 mL	0.06286 mL	0.06268 mL	0.94003 mL	0.02500 mL	8.203	0.09830	0.97701
17:58.0	Data point 26	0.55997 mL	0.06286 mL	0.06277 mL	0.94003 mL	0.02500 mL	8.545	0.10075	0.99009
18:45.2	Data point 27	0.55997 mL	0.06286 mL	0.06286 mL	0.94003 mL	0.02500 mL	9.000	0.10031	0.98035
19:34.8	Data point 28	0.55997 mL	0.06286 mL	0.06296 mL	0.94003 mL	0.02500 mL	9.383	0.09848	0.97128
20:13.6	Data point 29	0.55997 mL	0.06286 mL	0.06305 mL	0.94003 mL	0.02500 mL	9.663	0.09650	0.97049
20:48.1	Data point 30	0.55997 mL	0.06286 mL	0.06317 mL	0.94003 mL	0.02500 mL	9.917	0.09215	0.95609
21:14.1	Data point 31	0.55997 mL	0.06286 mL	0.06331 mL	0.94003 mL	0.02500 mL	10.127	0.08765	0.97340
21:30.7	Data point 32	0.55997 mL	0.06286 mL	0.06352 mL	0.94003 mL	0.02500 mL	10.360	0.04234	0.95725
22:02.5	Data point 33	0.55997 mL	0.06286 mL	0.06392 mL	0.94003 mL	0.02500 mL	10.561	0.03649	0.68615
22:29.1	Data point 34	0.55997 mL	0.06286 mL	0.06449 mL	0.94003 mL	0.02500 mL	10.754	0.01715	0.95910
22:45.7	Data point 35	0.55997 mL	0.06286 mL	0.06533 mL	0.94003 mL	0.02500 mL	10.951	0.01014	0.78929
23:02.3	Data point 36	0.55997 mL	0.06286 mL	0.06667 mL	0.94003 mL	0.02500 mL	11.143	0.00494	0.47749
23:18.9	Data point 37	0.55997 mL	0.06286 mL	0.06874 mL	0.94003 mL	0.02500 mL	11.309	0.00324	0.49907
23:45.8	Data point 38	0.55997 mL	0.06286 mL	0.07135 mL	0.94003 mL	0.02500 mL	11.501	0.00097	0.04113
24:02.5	Data point 39	0.55997 mL	0.06286 mL	0.07601 mL	0.94003 mL	0.02500 mL	11.691	0.00223	0.05553
24:19.3	Data point 40	0.55997 mL	0.06286 mL	0.08323 mL	0.94003 mL	0.02500 mL	11.887	0.00091	0.00476
24:36.0	Data point 41	0.55997 mL	0.06286 mL	0.09210 mL	0.94003 mL	0.02500 mL	12.041	0.00269	0.46279
26:19.4	Reference spectrum								
27:25.5	Data point 43	0.83996 mL	0.16197 mL	0.09212 mL	0.94003 mL	0.02500 mL	1.978	-0.08350	0.97007
27:53.0	Data point 44	0.83996 mL	0.16197 mL	0.11825 mL	0.94003 mL	0.02500 mL	2.174	-0.00087	0.04268
28:09.8	Data point 45	0.83996 mL	0.16197 mL	0.13351 mL	0.94003 mL	0.02500 mL	2.367	0.00247	0.09526
28:26.6	Data point 46	0.83996 mL	0.16197 mL	0.14337 mL	0.94003 mL	0.02500 mL	2.565	-0.00039	0.00634
28:43.4	Data point 47	0.83996 mL	0.16197 mL	0.14967 mL	0.94003 mL	0.02500 mL	2.764	-0.01019	0.43089
28:60.0	Data point 48	0.83996 mL	0.16197 mL	0.15374 mL	0.94003 mL	0.02500 mL	2.952	0.01826	0.65537
29:16.6	Data point 49	0.83996 mL	0.16197 mL	0.15635 mL	0.94003 mL	0.02500 mL	3.141	0.01355	0.91648



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 Instrument ID: **T311053**

**Events (continued)**

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
29:33.2	Data point 50	0.83996 mL	0.16197 mL	0.15804 mL	0.94003 mL	0.02500 mL	3.372	0.01291	0.83401	0.00
30:05.0	Data point 51	0.83996 mL	0.16197 mL	0.15906 mL	0.94003 mL	0.02500 mL	3.579	0.02189	0.96607	0.00
30:21.4	Data point 52	0.83996 mL	0.16197 mL	0.15967 mL	0.94003 mL	0.02500 mL	3.799	0.02736	0.95534	0.00
30:38.0	Data point 53	0.83996 mL	0.16197 mL	0.16004 mL	0.94003 mL	0.02500 mL	3.978	0.04177	0.97852	0.00
30:54.5	Data point 54	0.83996 mL	0.16197 mL	0.16030 mL	0.94003 mL	0.02500 mL	4.129	0.04988	0.94904	0.00
31:16.1	Data point 55	0.83996 mL	0.16197 mL	0.16061 mL	0.94003 mL	0.02500 mL	4.463	0.09865	0.97034	0.00
31:51.3	Data point 56	0.83996 mL	0.16197 mL	0.16082 mL	0.94003 mL	0.02500 mL	4.720	0.09756	0.97113	0.00
32:28.0	Data point 57	0.83996 mL	0.16197 mL	0.16094 mL	0.94003 mL	0.02500 mL	5.065	0.09518	0.94091	0.00
33:04.5	Data point 58	0.83996 mL	0.16197 mL	0.16101 mL	0.94003 mL	0.02500 mL	5.418	0.09946	0.98584	0.00
33:55.6	Data point 59	0.83996 mL	0.16197 mL	0.16108 mL	0.94003 mL	0.02500 mL	5.883	0.09865	0.96154	0.00
34:44.3	Data point 60	0.83996 mL	0.16197 mL	0.16115 mL	0.94003 mL	0.02500 mL	6.233	0.10040	0.98267	0.00
35:25.9	Data point 61	0.83996 mL	0.16197 mL	0.16124 mL	0.94003 mL	0.02500 mL	6.571	0.09858	0.98384	0.00
35:51.5	Data point 62	0.83996 mL	0.16197 mL	0.16134 mL	0.94003 mL	0.02500 mL	6.819	0.09621	0.97878	0.00
36:21.8	Data point 63	0.83996 mL	0.16197 mL	0.16145 mL	0.94003 mL	0.02500 mL	7.068	0.09852	0.97337	0.00
36:57.5	Data point 64	0.83996 mL	0.16197 mL	0.16157 mL	0.94003 mL	0.02500 mL	7.309	0.09522	0.97045	0.00
37:34.0	Data point 65	0.83996 mL	0.16197 mL	0.16169 mL	0.94003 mL	0.02500 mL	7.558	0.09456	0.95667	0.00
38:13.2	Data point 66	0.83996 mL	0.16197 mL	0.16181 mL	0.94003 mL	0.02500 mL	7.817	0.09971	0.98931	0.00
38:59.4	Data point 67	0.83996 mL	0.16197 mL	0.16192 mL	0.94003 mL	0.02500 mL	8.097	0.09824	0.98504	0.00
39:46.7	Data point 68	0.83996 mL	0.16197 mL	0.16204 mL	0.94003 mL	0.02500 mL	8.498	0.09675	0.97066	0.00
40:34.3	Data point 69	0.83996 mL	0.16197 mL	0.16214 mL	0.94003 mL	0.02500 mL	8.860	0.09546	0.94457	0.00
41:15.5	Data point 70	0.83996 mL	0.16197 mL	0.16223 mL	0.94003 mL	0.02500 mL	9.199	0.09471	0.96373	0.00
41:56.0	Data point 71	0.83996 mL	0.16197 mL	0.16232 mL	0.94003 mL	0.02500 mL	9.457	0.09619	0.98303	0.00
42:25.2	Data point 72	0.83996 mL	0.16197 mL	0.16244 mL	0.94003 mL	0.02500 mL	9.666	0.09304	0.95434	0.00
42:48.8	Data point 73	0.83996 mL	0.16197 mL	0.16261 mL	0.94003 mL	0.02500 mL	9.884	0.07358	0.96770	0.00
43:10.3	Data point 74	0.83996 mL	0.16197 mL	0.16282 mL	0.94003 mL	0.02500 mL	10.082	0.05143	0.94953	0.00
43:42.1	Data point 75	0.83996 mL	0.16197 mL	0.16322 mL	0.94003 mL	0.02500 mL	10.278	0.03259	0.96023	0.00
44:03.7	Data point 76	0.83996 mL	0.16197 mL	0.16399 mL	0.94003 mL	0.02500 mL	10.551	0.01201	0.90403	0.00
44:35.5	Data point 77	0.83996 mL	0.16197 mL	0.16498 mL	0.94003 mL	0.02500 mL	10.761	0.00289	0.23732	0.00
45:07.2	Data point 78	0.83996 mL	0.16197 mL	0.16620 mL	0.94003 mL	0.02500 mL	10.980	-0.00281	0.37825	0.00
45:23.7	Data point 79	0.83996 mL	0.16197 mL	0.16837 mL	0.94003 mL	0.02500 mL	11.206	-0.00388	0.43580	0.00
45:40.3	Data point 80	0.83996 mL	0.16197 mL	0.17197 mL	0.94003 mL	0.02500 mL	11.393	-0.00805	0.67686	0.00
45:56.9	Data point 81	0.83996 mL	0.16197 mL	0.17747 mL	0.94003 mL	0.02500 mL	11.595	-0.00505	0.50431	0.00
46:13.6	Data point 82	0.83996 mL	0.16197 mL	0.18624 mL	0.94003 mL	0.02500 mL	11.790	-0.00646	0.54188	0.00
46:30.6	Data point 83	0.83996 mL	0.16197 mL	0.20007 mL	0.94003 mL	0.02500 mL	11.982	-0.00387	0.35786	0.00
46:47.3	Data point 84	0.83996 mL	0.16197 mL	0.20640 mL	0.94003 mL	0.02500 mL	12.049	-0.00571	0.64544	0.00
48:43.8	Reference spectrum									
51:00.1	Data point 86	1.54998 mL	0.30635 mL	0.20642 mL	0.94003 mL	0.02500 mL	1.991	0.03841	0.87553	0.00
51:33.0	Data point 87	1.54998 mL	0.30635 mL	0.23857 mL	0.94003 mL	0.02500 mL	2.184	-0.03480	0.94123	0.00
51:50.1	Data point 88	1.54998 mL	0.30635 mL	0.25847 mL	0.94003 mL	0.02500 mL	2.381	-0.09786	0.93944	0.00
52:07.9	Data point 89	1.54998 mL	0.30635 mL	0.27159 mL	0.94003 mL	0.02500 mL	2.582	-0.07525	0.74838	0.00
52:27.3	Data point 90	1.54998 mL	0.30635 mL	0.27980 mL	0.94003 mL	0.02500 mL	2.782	-0.09219	0.87508	0.00
53:01.3	Data point 91	1.54998 mL	0.30635 mL	0.28504 mL	0.94003 mL	0.02500 mL	2.951	0.00100	0.02437	0.00
53:23.1	Data point 92	1.54998 mL	0.30635 mL	0.28937 mL	0.94003 mL	0.02500 mL	3.184	-0.09569	0.89828	0.00
53:55.5	Data point 93	1.54998 mL	0.30635 mL	0.29146 mL	0.94003 mL	0.02500 mL	3.376	-0.00395	0.29874	0.00
54:12.0	Data point 94	1.54998 mL	0.30635 mL	0.29278 mL	0.94003 mL	0.02500 mL	3.570	-0.09273	0.89362	0.00
54:28.4	Data point 95	1.54998 mL	0.30635 mL	0.29365 mL	0.94003 mL	0.02500 mL	3.752	-0.09248	0.83398	0.00
54:45.5	Data point 96	1.54998 mL	0.30635 mL	0.29421 mL	0.94003 mL	0.02500 mL	3.910	-0.08663	0.86878	0.00
55:12.8	Data point 97	1.54998 mL	0.30635 mL	0.29534 mL	0.94003 mL	0.02500 mL	4.174	-0.08811	0.87064	0.00
55:35.5	Data point 98	1.54998 mL	0.30635 mL	0.29577 mL	0.94003 mL	0.02500 mL	4.539	-0.09290	0.86960	0.00
56:05.7	Data point 99	1.54998 mL	0.30635 mL	0.29605 mL	0.94003 mL	0.02500 mL	4.819	-0.02689	0.46322	0.00
56:32.2	Data point 100	1.54998 mL	0.30635 mL	0.29617 mL	0.94003 mL	0.02500 mL	5.070	0.09888	0.99100	0.00
57:03.7	Data point 101	1.54998 mL	0.30635 mL	0.29626 mL	0.94003 mL	0.02500 mL	5.397	0.09694	0.96285	0.00
57:32.8	Data point 102	1.54998 mL	0.30635 mL	0.29633 mL	0.94003 mL	0.02500 mL	5.617	0.06814	0.72545	0.00
57:59.5	Data point 103	1.54998 mL	0.30635 mL	0.29640 mL	0.94003 mL	0.02500 mL	5.896	0.04399	0.81393	0.00
58:20.9	Data point 104	1.54998 mL	0.30635 mL	0.29650 mL	0.94003 mL	0.02500 mL	6.144	-0.08249	0.80902	0.00
58:46.1	Data point 105	1.54998 mL	0.30635 mL	0.29657 mL	0.94003 mL	0.02500 mL	6.343	-0.09246	0.91831	0.00
59:09.1	Data point 106	1.54998 mL	0.30635 mL	0.29666 mL	0.94003 mL	0.02500 mL	6.544	-0.09305	0.96686	0.00

Sample name: **M06**  
 Assay name: **UV-metric psKa**  
 Assay ID: **17K-16011**  
 Filename: **C:\Sirius\_T3\Mehtap\20171116\_exp18\_pKa\17K-16011\_M06\_UV-metric psKa.t3r**

Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Events (continued)

Time	Event	Water	Acid	Base	Methanol	Buffer	pH	dpH/dt	pH R-squared	pH SD
59:30.7	Data point 107	1.54998 mL	0.30635 mL	0.29673 mL	0.94003 mL	0.02500 mL	6.680	-0.04606	0.60999	0.0029
59:52.2	Data point 108	1.54998 mL	0.30635 mL	0.29683 mL	0.94003 mL	0.02500 mL	6.885	-0.09634	0.97572	0.0048
1:00:20.4	Data point 109	1.54998 mL	0.30635 mL	0.29692 mL	0.94003 mL	0.02500 mL	7.083	-0.06951	0.97160	0.0034
1:00:46.8	Data point 110	1.54998 mL	0.30635 mL	0.29701 mL	0.94003 mL	0.02500 mL	7.281	-0.01955	0.69708	0.0011
1:01:18.6	Data point 111	1.54998 mL	0.30635 mL	0.29713 mL	0.94003 mL	0.02500 mL	7.510	0.04801	0.70845	0.0028
1:01:50.2	Data point 112	1.54998 mL	0.30635 mL	0.29725 mL	0.94003 mL	0.02500 mL	7.811	0.08676	0.90651	0.0045
1:02:24.0	Data point 113	1.54998 mL	0.30635 mL	0.29734 mL	0.94003 mL	0.02500 mL	8.157	0.09029	0.91956	0.0046
1:02:56.9	Data point 114	1.54998 mL	0.30635 mL	0.29741 mL	0.94003 mL	0.02500 mL	8.509	0.09349	0.95432	0.0047
1:03:32.5	Data point 115	1.54998 mL	0.30635 mL	0.29748 mL	0.94003 mL	0.02500 mL	8.871	0.08847	0.89436	0.0046
1:04:01.1	Data point 116	1.54998 mL	0.30635 mL	0.29755 mL	0.94003 mL	0.02500 mL	9.104	0.08787	0.87388	0.0046
1:04:22.5	Data point 117	1.54998 mL	0.30635 mL	0.29762 mL	0.94003 mL	0.02500 mL	9.302	0.03414	0.81033	0.0018
1:04:44.2	Data point 118	1.54998 mL	0.30635 mL	0.29772 mL	0.94003 mL	0.02500 mL	9.499	0.00728	0.46472	0.0005
1:05:00.6	Data point 119	1.54998 mL	0.30635 mL	0.29786 mL	0.94003 mL	0.02500 mL	9.770	-0.07693	0.85364	0.0041
1:05:17.1	Data point 120	1.54998 mL	0.30635 mL	0.29800 mL	0.94003 mL	0.02500 mL	9.953	-0.07541	0.82043	0.0041
1:05:33.5	Data point 121	1.54998 mL	0.30635 mL	0.29819 mL	0.94003 mL	0.02500 mL	10.146	-0.09516	0.90059	0.0049
1:06:00.2	Data point 122	1.54998 mL	0.30635 mL	0.29873 mL	0.94003 mL	0.02500 mL	10.395	-0.04385	0.87593	0.0023
1:06:16.8	Data point 123	1.54998 mL	0.30635 mL	0.29977 mL	0.94003 mL	0.02500 mL	10.645	-0.09213	0.89422	0.0048
1:06:49.0	Data point 124	1.54998 mL	0.30635 mL	0.30148 mL	0.94003 mL	0.02500 mL	10.837	-0.01807	0.91982	0.0009
1:07:05.7	Data point 125	1.54998 mL	0.30635 mL	0.30416 mL	0.94003 mL	0.02500 mL	11.026	-0.08591	0.89590	0.0044
1:07:22.2	Data point 126	1.54998 mL	0.30635 mL	0.30835 mL	0.94003 mL	0.02500 mL	11.256	-0.08853	0.91460	0.0045
1:07:39.4	Data point 127	1.54998 mL	0.30635 mL	0.31543 mL	0.94003 mL	0.02500 mL	11.468	-0.09433	0.92673	0.0048
1:07:56.3	Data point 128	1.54998 mL	0.30635 mL	0.32695 mL	0.94003 mL	0.02500 mL	11.682	-0.08409	0.91335	0.0043
1:08:13.3	Data point 129	1.54998 mL	0.30635 mL	0.34586 mL	0.94003 mL	0.02500 mL	11.894	-0.08478	0.92156	0.0043
1:08:30.4	Data point 130	1.54998 mL	0.30635 mL	0.36896 mL	0.94003 mL	0.02500 mL	12.060	-0.08156	0.96400	0.0041
1:10:01.2	Assay volumes	1.54998 mL	0.47368 mL	0.36896 mL	0.94003 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	3			
Minimum pH	2.000			
Maximum pH	12.000			
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	Yes			
Cosolvent type	Methanol			
Cosolvent volume	0.94 mL			
Cosolvent added	Automatic			
ISA water volume	0.56 mL			
Water added	Automatic			
After water addition, stir for	5 seconds			

Sample name: **M06**  
 Assay name: **UV-metric psKa**  
 Assay ID: **17K-16011**  
 Filename: **C:\Sirius\_T3\Mehtap\20171116\_exp18\_pKa\17K-16011\_M06\_UV-metric psKa.t3r**

Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## Assay Settings (continued)

Setting	Value	Original Value	Date/Time changed	Imported from
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>Titration 2</b>				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.28 mL			
Additional water added	Automatic			
After pH adjust stir for	10 seconds			
<b>Titration 3</b>				
Titrate from	Low to high pH			
Additional cosolvent volume	0.00 mL			
Add additional water	0.71 mL			
Additional water added	Automatic			
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	0.116	11/16/2017 12:23:06 PM	C:\Sirius_T3\17K-16010_Blank standardisation.t3r
Four-Plus S	1.0003	11/16/2017 12:23:06 PM	C:\Sirius_T3\17K-16010_Blank standardisation.t3r
Four-Plus jH	-0.3	11/16/2017 12:23:06 PM	C:\Sirius_T3\17K-16010_Blank standardisation.t3r
Four-Plus jOH	0.0	11/16/2017 12:23:06 PM	C:\Sirius_T3\17K-16010_Blank standardisation.t3r
Base concentration factor	1.008	11/16/2017 12:23:06 PM	C:\Sirius_T3\KOH17K09.t3r
Acid concentration factor	1.007	11/16/2017 12:23:06 PM	C:\Sirius_T3\17K-16010_Blank standardisation.t3r

Sample name: **M06**  
 Assay name: **UV-metric psKa**  
 Assay ID: **17K-16011**  
 Filename: **C:\Sirius\_T3\Mehtap\20171116\_exp18\_pKa\17K-16011\_M06\_UV-metric psKa.t3r**

Experiment start time: **11/16/2017 12:23:06 PM**  
 Analyst: **Dorothy Levorse**  
 Instrument ID: **T311053**

## 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)	10-10-2017	11/8/2017 11:33:30 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)	11-8-17	11/8/2017 11:32:21 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)	10-30-17	10/30/2017 8:01:46 AM
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	11/16/2017 9:31:07 AM
Port B	Cyclohexane		10/19/2017 2:11:05 PM
Port C	MeCN (50%, 0.15 M KCl)	10-30-17	10/30/2017 8:02:00 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		11/8/2017 11:32:27 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	10/13/2017 7:46:59 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	-5.74 mV		11/16/2017 12:23:34 PM
Filling solution	3M KCl	KCL095	11/16/2017 9:28:10 AM
Liquids			
Wash 1	50% IPA:50% Water		11/16/2017 9:31:35 AM
Wash 2	0.5% Triton X-100 in H2O		11/16/2017 9:31:38 AM
Buffer position 1	pH7 Wash		11/16/2017 9:31:40 AM
Buffer position 2	pH 7		11/16/2017 9:31:42 AM
Storage position			11/16/2017 9:32:48 AM
Wash water	7.3e+003 mL	11-10-17	11/10/2017 10:14:37 AM
Waste	1e+004 mL		10/13/2017 8:58:05 AM
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	535:10:13		11/23/2010 11:22:28 AM
Calibrated on	11/8/2017 1:14:37 PM		



## Assay Settings

Sample name: **M06** Experiment start time: **11/16/2017 12:23:06 PM**  
Assay name: **UV-metric psKa** Analyst: **Dorothy Levorse**  
Assay ID: **17K-16011** Instrument ID: **T311053**  
Filename: **C:\Sirius\_T3\Mehtap\20171116\_exp18\_pKa\17K-16011\_M06\_UV-metric psKa.t3r**

### Instrument Settings (continued)

Setting	Value	Batch Id	Install date
Integration time	10		
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

### Tray Information

Title  
Location A1