# Yasuda-Shedlovsky Result

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

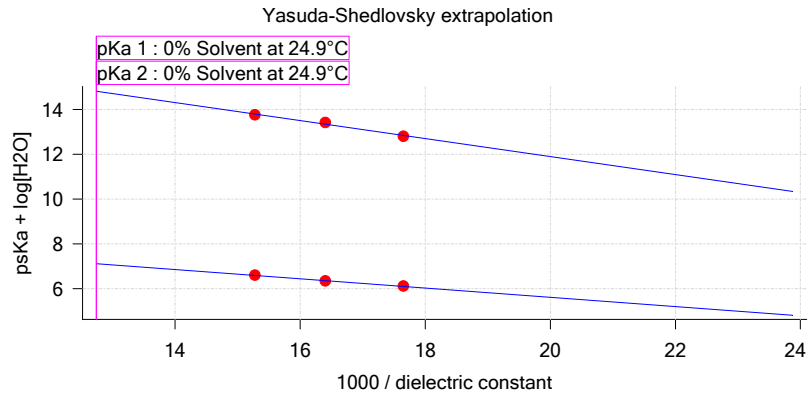
## Yasuda-Shedlovsky result

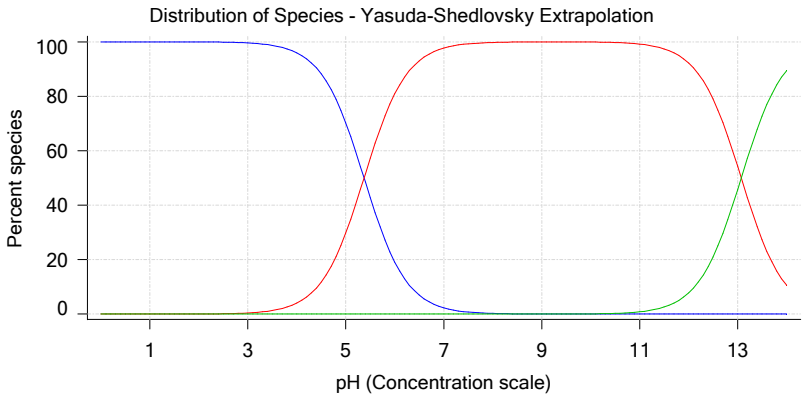
| Extrapolation type | pKa 0% | SD | Intercept | Slope | R² | Ionic strength | Temperature |
|---|---|---|---|---|---|---|---|
| Yasuda-Shedlovsky | 5.37 | ±0.06 | 9.74 | -206.2268 | 0.9942 | 0.165 M | 24.9°C |
| Yasuda-Shedlovsky | 13.07 | ±0.21 | 19.95 | -402.7639 | 0.9819 | 0.165 M | 24.9°C |

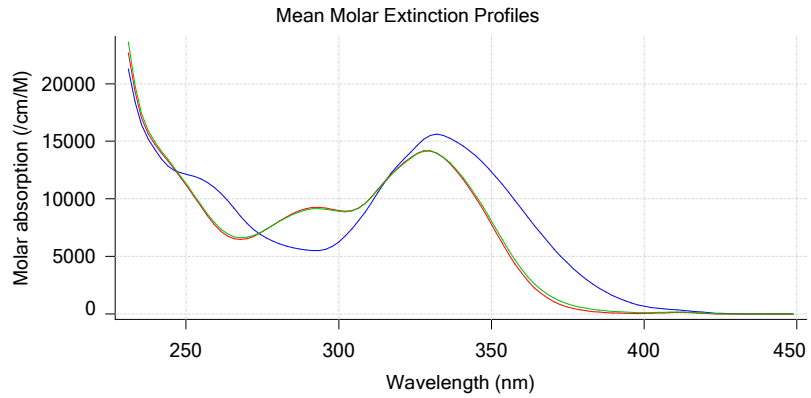
## Component assay results

| Titration | Methanol weight% | Direction | Result type | Dielectric constant | [H2O] | Ionic strength | Temperature | psKa 1 | psKa 2 |
|---|---|---|---|---|---|---|---|---|---|
| 17I-16014 Points 4 to 42 | 49.43 % | Up | UV-metric pKa | 56.7 | 24.7 M | 0.157 M | 24.9°C ☑ | 4.72 ☑ | 11.41 |
| 17I-16014 Points 44 to 86 | 40.08 % | Up | UV-metric pKa | 61.0 | 30.0 M | 0.166 M | 24.9°C ☑ | 4.86 ☑ | 11.94 |
| 17I-16014 Points 88 to 126 | 30.19 % | Up | UV-metric pKa | 65.4 | 35.8 M | 0.172 M | 24.9°C ☑ | 5.05 ☑ | 12.20 |

## Graphs




# UV-metric psKa Titration 1 of 3 17I-16014 Points 4 to 42

## Results

| | |
|---|---|
| pKa 1 | **4.72** |
| pKa 2 | **11.41** |
| RMSD | **0.003 0.068 0.068** |
| Chi squared | **0.2605** |
| PCA calculated number of pKas | **2** |
| Average ionic strength | **0.157 M** |
| Average temperature | **24.9°C** |
| Analyte concentration range | **52.3 µM to 49.2 µM** |
| Methanol weight % | **49.4 %** |
| Dielectric constant | **56.7** |
| Water concentration | **24.7 M** |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Results (continued)

Number of pKas source **Manual (2)**
Wavelength clipping **232.8 nm to 450.0 nm**
pH clipping **1.459 to 12.511**

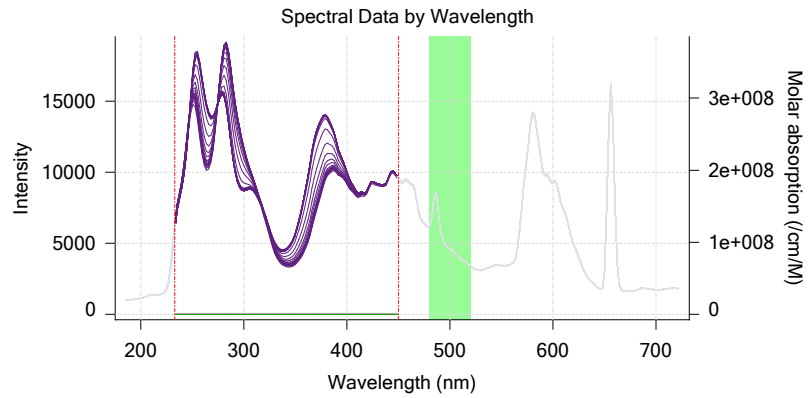
## Warnings and errors

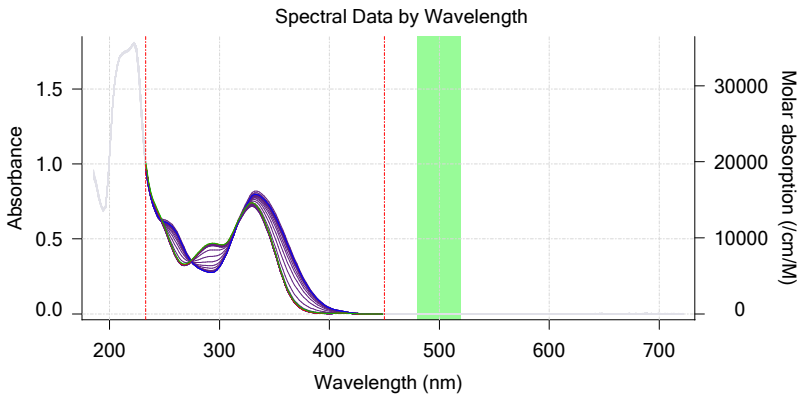
Errors None
Warnings RMSD exceeds warning threshold

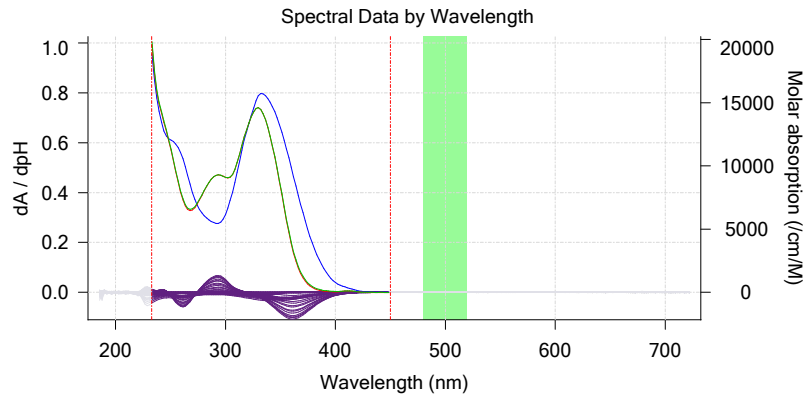
## Assay Settings

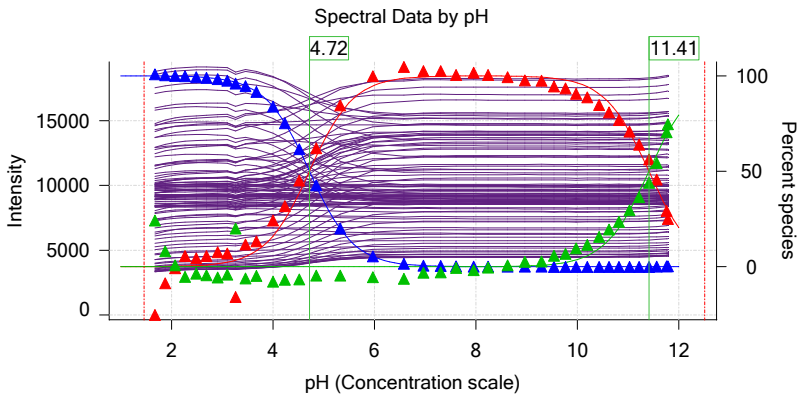
| Setting | Value | Original Value | Date/Time changed | Imported from |
|---|---|---|---|---|
| Buffer in use | Yes | | | |
| Buffer type | Phosphate Buffer | | | |
| ***Assay Medium*** | | | | |
| Volume of buffer introduced | 0.025000 mL | | | |
| Add buffer manually | Manual | | | |

## Graphs



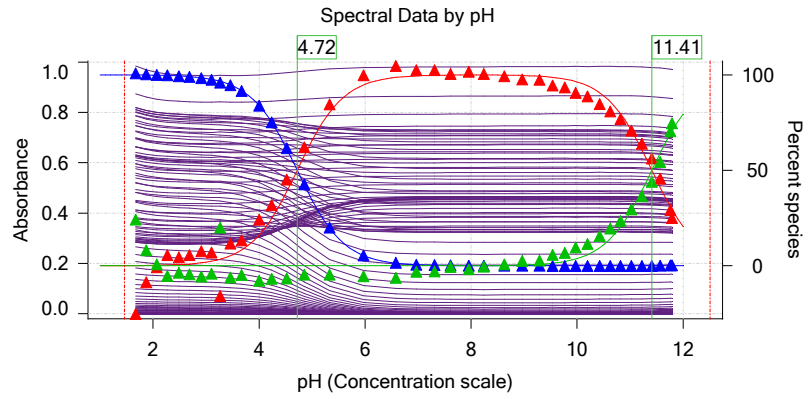



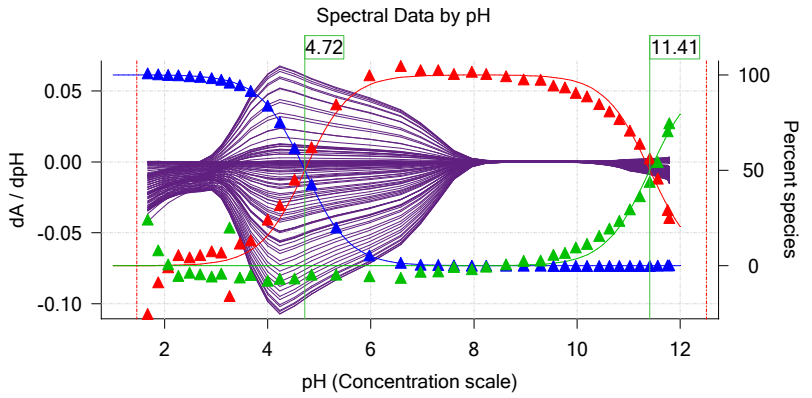



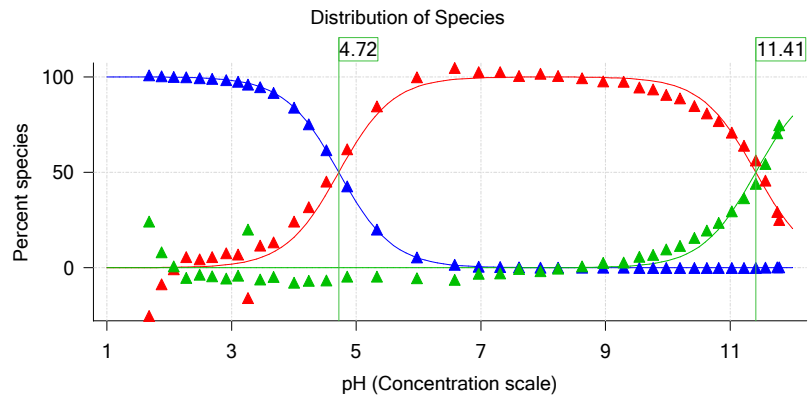
Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

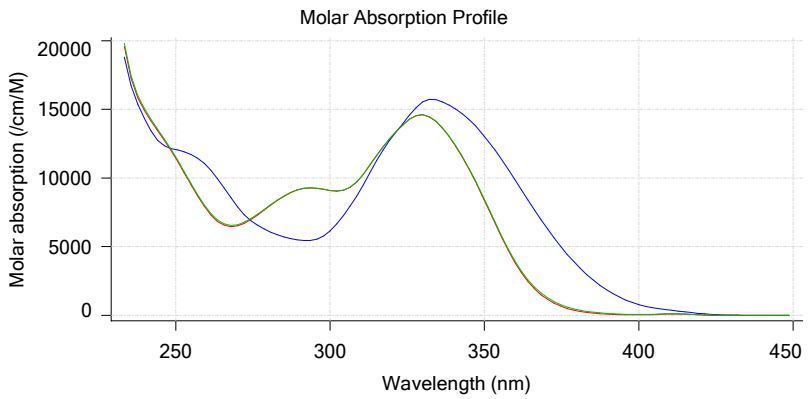
Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

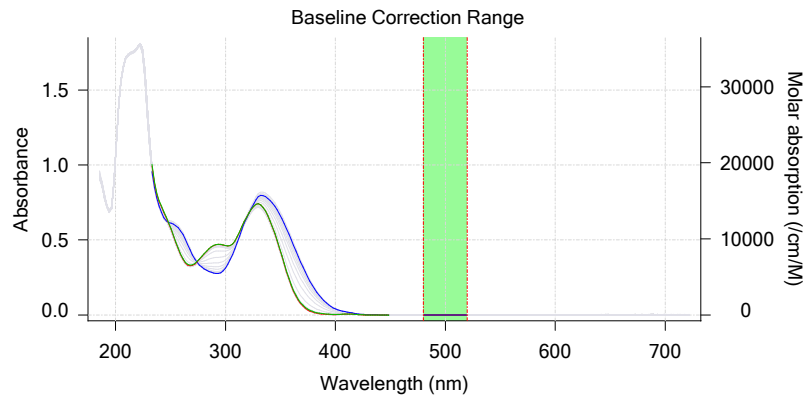
## Graphs (continued)

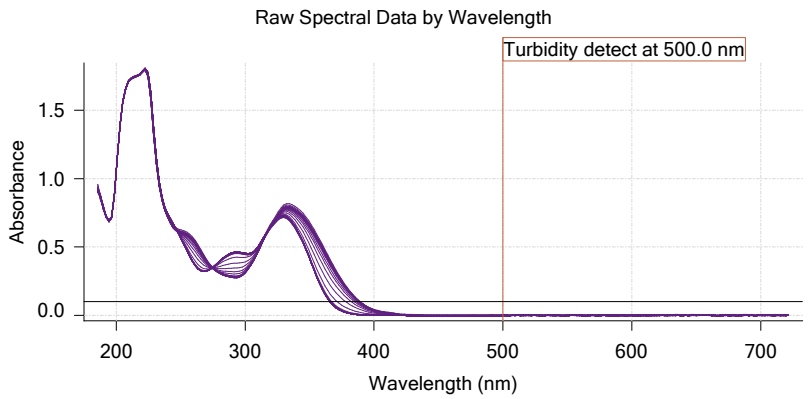



# UV-metric psKa Titration 2 of 3 17I-16014 Points 44 to 86

## Results

| | |
|---|---|
| pKa 1 | **4.86** |
| pKa 2 | **11.94** |
| RMSD | **0.001 0.053 0.053** |
| Chi squared | **0.8936** |
| PCA calculated number of pKas | **2** |
| Average ionic strength | **0.166 M** |
| Average temperature | **24.9°C** |
| Analyte concentration range | **42.9 µM to 40.6 µM** |
| Methanol weight % | **40.1 %** |
| Dielectric constant | **61.0** |
| Water concentration | **30.0 M** |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Results (continued)

Number of pKas source **Manual (2)**
Wavelength clipping **230.0 nm to 450.0 nm**
pH clipping **1.498 to 12.525**

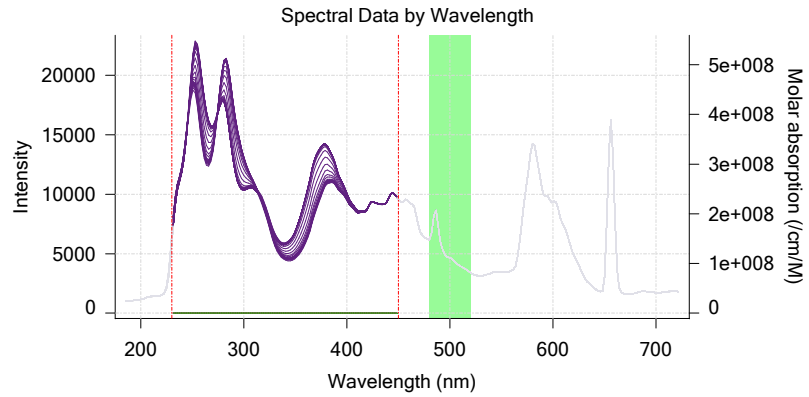
## Warnings and errors

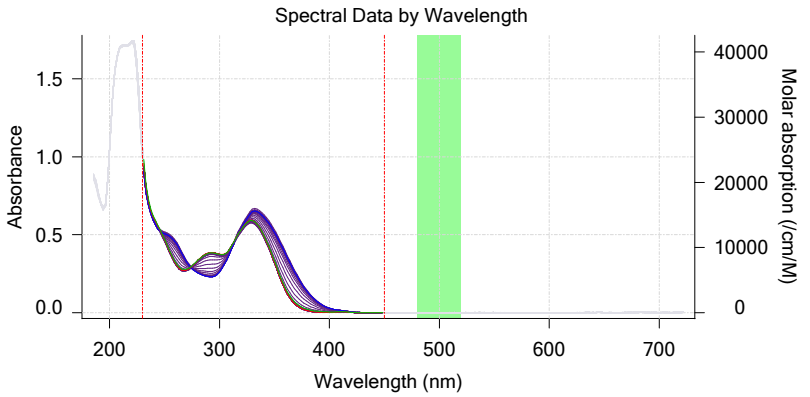
Errors None
Warnings RMSD exceeds warning threshold

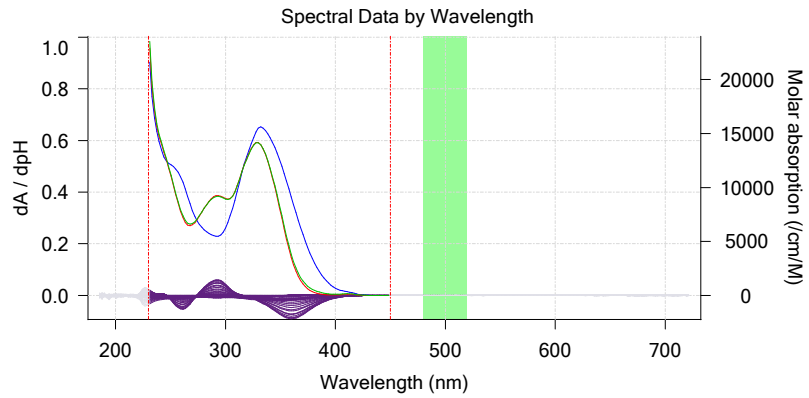
## Assay Settings

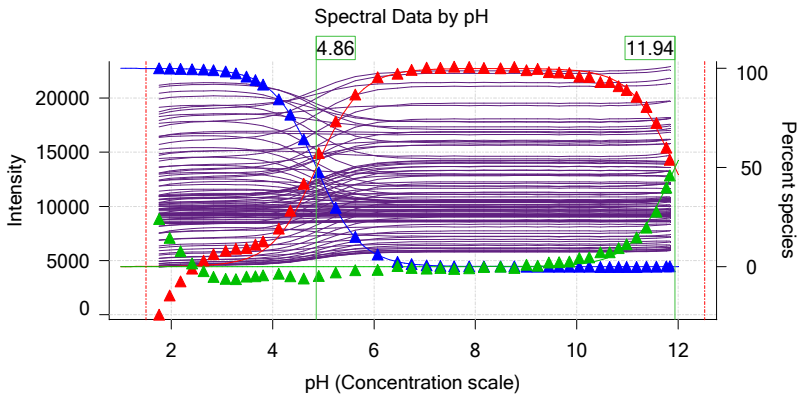
| Setting | Value | Original Value | Date/Time changed | Imported from |
|---|---|---|---|---|
| Buffer in use | Yes | | | |
| Buffer type | Phosphate Buffer | | | |
| ***Assay Medium*** | | | | |
| Volume of buffer introduced | 0.025000 mL | | | |
| Add buffer manually | Manual | | | |

## Graphs



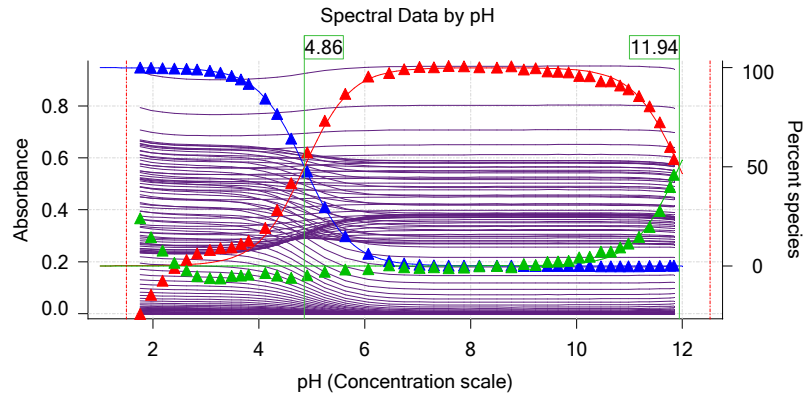



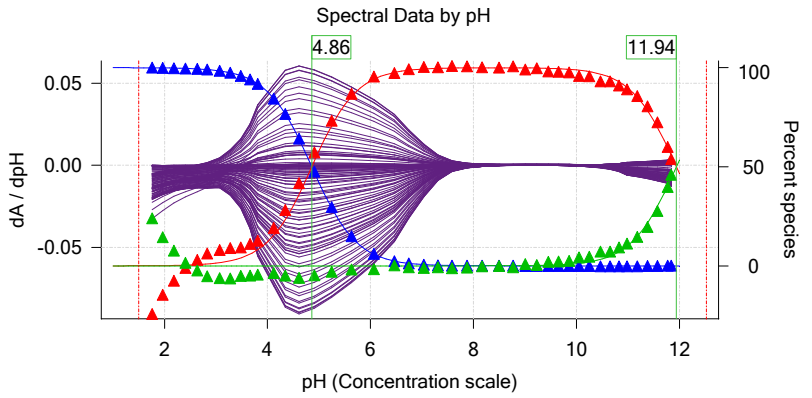



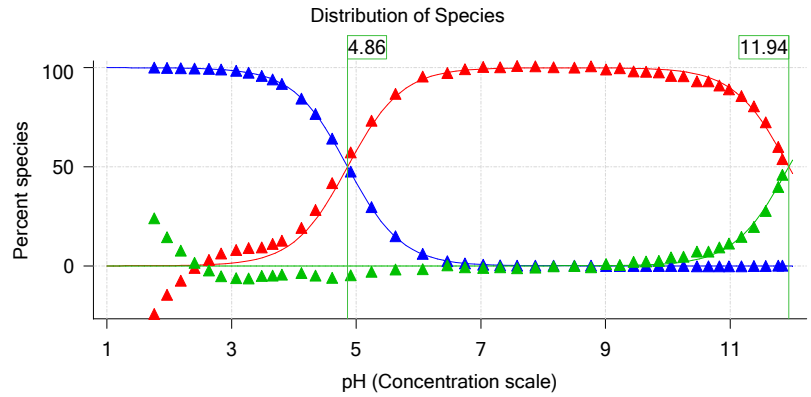
Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

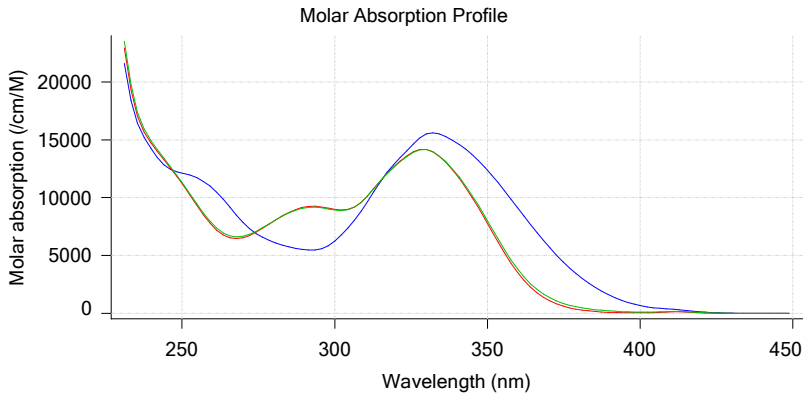
Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

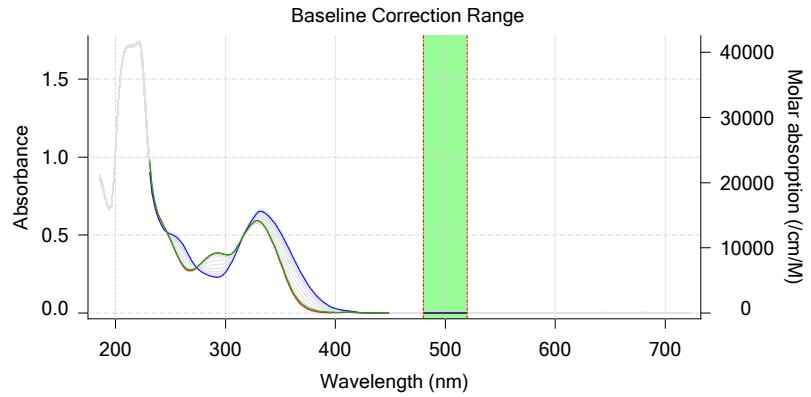
## Graphs (continued)

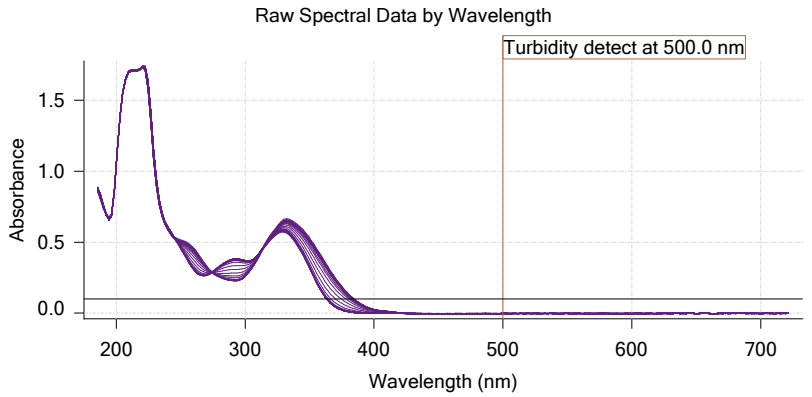



## UV-metric psKa Titration 3 of 3 17I-16014 Points 88 to 126

### Results

| | |
|---|---|
| pKa 1 | **5.05** |
| pKa 2 | **12.20** |
| RMSD | **0.001 0.019 0.019** |
| Chi squared | **0.3688** |
| PCA calculated number of pKas | **2** |
| Average ionic strength | **0.172 M** |
| Average temperature | **24.9°C** |
| Analyte concentration range | **33.1 µM to 31.3 µM** |
| Methanol weight % | **30.2 %** |
| Dielectric constant | **65.4** |
| Water concentration | **35.8 M** |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Results (continued)

Number of pKas source **Manual (2)**
Wavelength clipping **230.0 nm to 450.0 nm**
pH clipping **1.493 to 12.527**

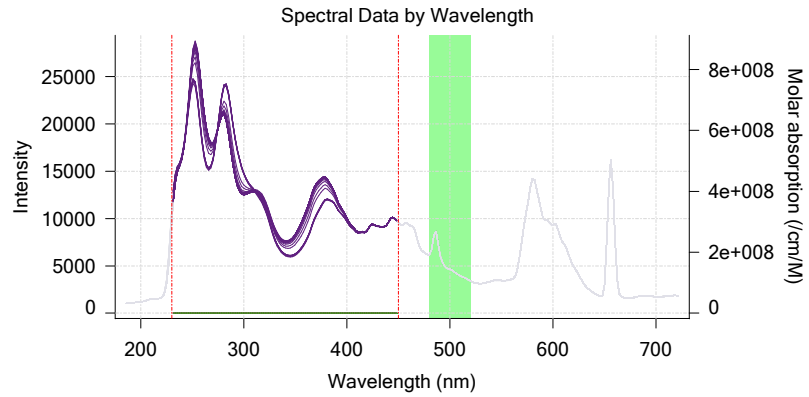
## Warnings and errors

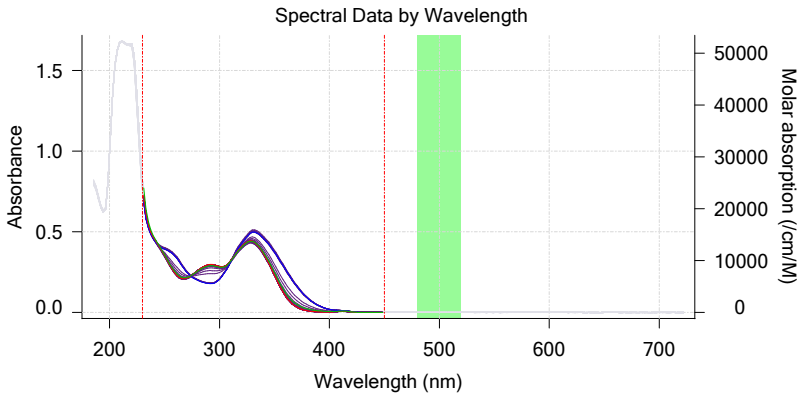
Errors None
Warnings None

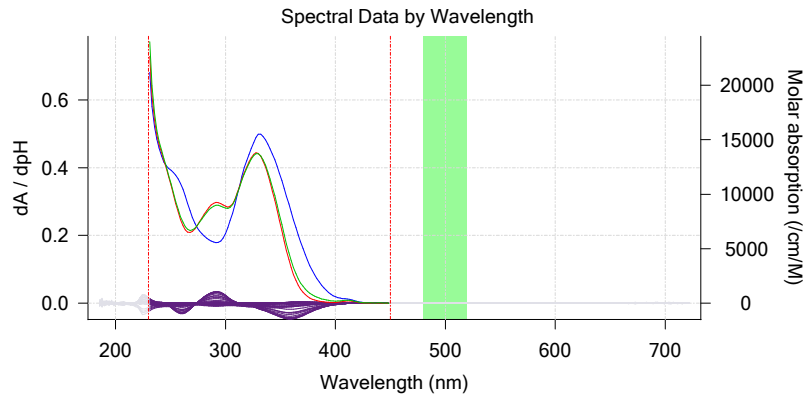
## Assay Settings

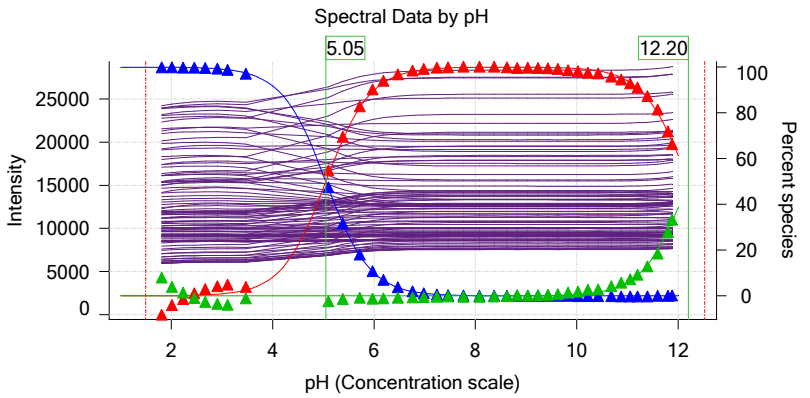
| Setting | Value | Original Value | Date/Time changed | Imported from |
|---|---|---|---|---|
| Buffer in use | Yes | | | |
| Buffer type | Phosphate Buffer | | | |
| ***Assay Medium*** | | | | |
| Volume of buffer introduced | 0.025000 mL | | | |
| Add buffer manually | Manual | | | |

## Graphs



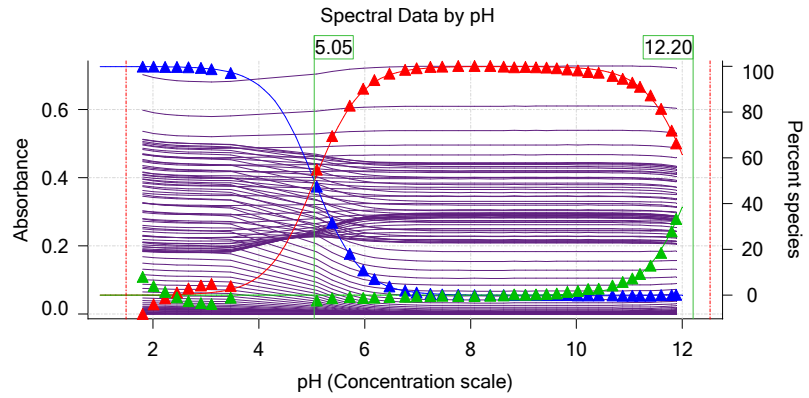



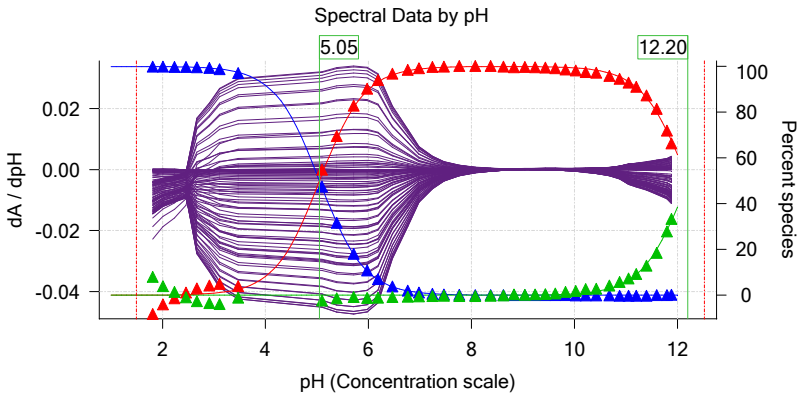



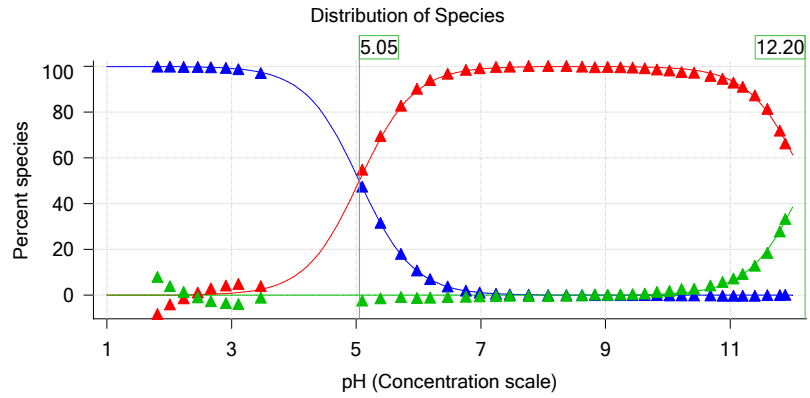
Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

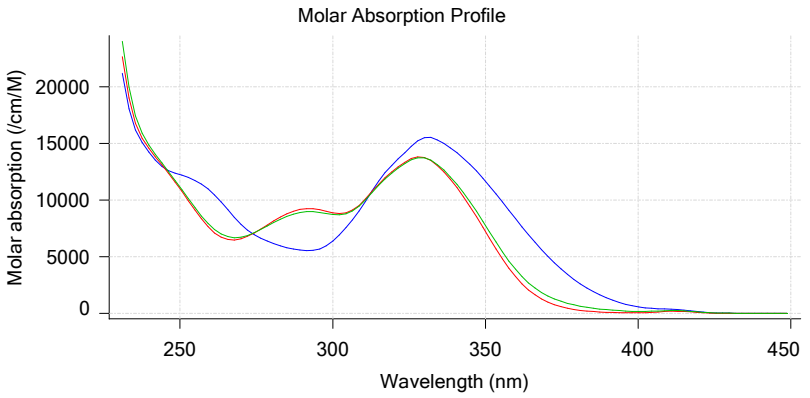
Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

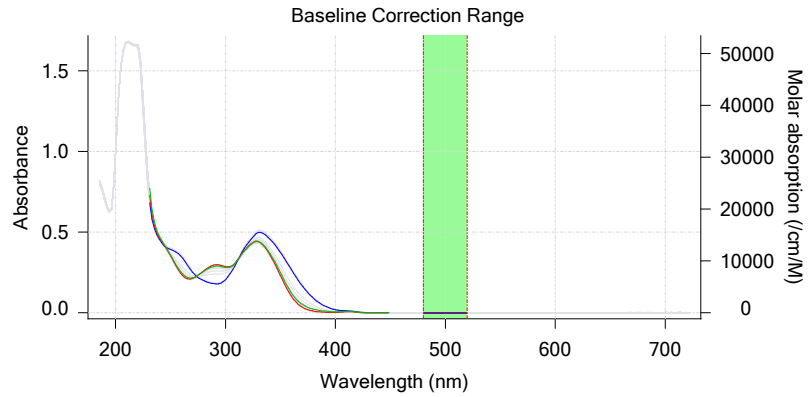
## Graphs (continued)

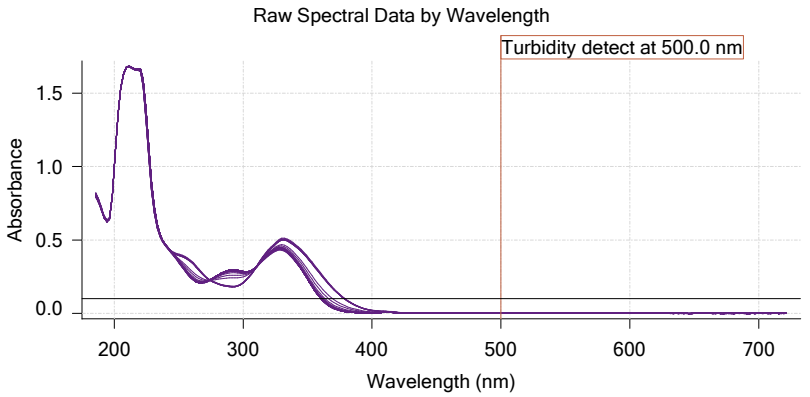



## Assay Model

| Settings | Value | Date/Time changed | Imported from |
|---|---|---|---|
| Sample name | M09 | 9/15/2017 4:43:48 PM | User entered value |
| Sample by | Volume | | Default value |
| Sample volume | 0.0020 mL | 9/15/2017 4:43:48 PM | User entered value |
| Solvent | DMSO | | Default value |
| Sample concentration | 0.041700 M | 9/15/2017 4:43:48 PM | User entered value |
| Solubility | Unknown | | Default value |
| Molecular weight | 287.74 | 9/15/2017 4:43:57 PM | User entered value |
| Individual pKa ionic environments | No | | Default value |
| Number of pKas | 2 | 9/15/2017 4:43:48 PM | User entered value |
| Sample is a | Base | 9/15/2017 4:43:48 PM | User entered value |
| pKa 1 | 2.90 | 9/15/2017 4:43:48 PM | User entered value |
| pKa 2 | 5.60 | 9/15/2017 4:43:48 PM | User entered value |
| logP (XH2 2+) | -10.00 | | Default value |
| logp (XH +) | -10.00 | | Default value |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Assay Model (continued)

| Settings | Value | Date/Time changed | Imported from |
|---|---|---|---|
| logP (neutral X) | -10.00 | 9/15/2017 4:43:48 PM | User entered value |
| Stoichiometry | 1.00000 | | Default value |
| Aprotic counterion name | Chloride | | From standards.xml file |
| Stoichiometry | 1.00 | | From standards.xml file |
| Charge per counterion | -1 | | From standards.xml file |

## Assay Settings

| Setting | Value | Original Value | Date/Time changed | Imported from |
|---|---|---|---|---|
| ***General Settings*** | | | | |
| Analyst name | Dorothy Levorse | | | |
| Separate reference vial | Yes | | | |
| ***Standard Experiment Settings*** | | | | |
| 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 | | | |
| ***Advanced General Settings*** | | | | |
| 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% | | | |
| ***Titrant Pre-Dose*** | | | | |
| Titrant pre-dose | None | | | |
| ***Assay Medium*** | | | | |
| Cosolvent in use | Yes | | | |
| Cosolvent type | Methanol | | | |
| Cosolvent volume | 1.15 mL | | | |
| Cosolvent added | Automatic | | | |
| ISA water volume | 0.35 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 | | | |
| ***Sample Sonication*** | | | | |
| Sonicate | No | | | |
| ***Sample Dissolution*** | | | | |
| Perform a dissolution stage | No | | | |
| ***Carbonate purge*** | | | | |
| Perform a carbonate purge | No | | | |
| ***Temperature Control*** | | | | |
| Wait for temperature | Yes | | | |
| Required start temperature | 25.0°C | | | |
| Acceptable deviation | 0.5°C | | | |
| Time to wait | 60 seconds | | | |
| Stir speed of | 15% | | | |
| ***Titration 1*** | | | | |
| Titrate from | Low to high pH | | | |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Assay Settings (continued)

| Setting | Value | Original Value | Date/Time changed | Imported from |
|---|---|---|---|---|
| Adjust to start pH | Yes | | | |
| After pH adjust stir for | 10 seconds | | | |
| ***Titration 2*** | | | | |
| Titrate from | Low to high pH | | | |
| Additional cosolvent volume | 0.00 mL | | | |
| Add additional water | 0.15 mL | | | |
| Additional water added | Automatic | | | |
| After pH adjust stir for | 10 seconds | | | |
| ***Titration 3*** | | | | |
| Titrate from | Low to high pH | | | |
| Additional cosolvent volume | 0.00 mL | | | |
| Add additional water | 0.34 mL | | | |
| Additional water added | Automatic | | | |
| After pH adjust stir for | 10 seconds | | | |
| ***Data Point Stability*** | | | | |
| 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 | | | |
| ***Experiment cleanup*** | | | | |
| 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 6:54:15 AM | C:\Sirius_T3\HCl17I15.t3r |
| Four-Plus S | 1.0006 | 9/16/2017 6:54:15 AM | C:\Sirius_T3\HCl17I15.t3r |
| Four-Plus jH | 0.7 | 9/16/2017 6:54:15 AM | C:\Sirius_T3\HCl17I15.t3r |
| Four-Plus jOH | -0.6 | 9/16/2017 6:54:15 AM | C:\Sirius_T3\HCl17I15.t3r |
| Base concentration factor | 1.015 | 9/16/2017 6:54:16 AM | C:\Sirius_T3\KOH17I11.t3r |
| Acid concentration factor | 1.003 | 9/16/2017 6:54:16 AM | C:\Sirius_T3\HCl17I15.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 |
| 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) | | |

Sample name: **M09**
Assay name: **UV-metric psKa**
Assay ID: **17I-16014**
Filename: **C:\Sirius_T3\Mehtap\20170915_exp03_uv_M01-M14\17I-16014_M09_UV-metric psKa.t3r**

Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Instrument Settings (continued)

| Setting | Value | Batch Id | Install date |
|---|---|---|---|
| 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 | -8.74 mV | | 9/16/2017 6:54:40 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% Trition X-100 in H20 | | 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 | 7.3e+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 | | |
| 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% | | |

Sample name: **M09**
Assay name: **UV-metric psKa**
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Experiment start time: **9/16/2017 6:54:16 AM**
Analyst: **Dorothy Levorse**
Instrument ID: **T311053**

## Instrument Settings (continued)

| Setting | Value | Batch Id | Install date |
|---|---|---|---|
| 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 F1