

Coordinate Sequence Table with Temperature Control with iChemExplorer Solubility Tool and Agilent Chemstation

Introduction:

The iChemExplorer ICEtray and ICE box add heating and stirring to the HPLC autosampler so chemistry can be done in the sample vial. Start by setting the vials in the ICEtray. Confirm that Agilent Chemstation on-line is up and running. Load the method to be used for sampling. Use the Solubility Tool to build a Sequence Table in Chemstation and a Heat Table in iChemExplorer that work together. Within twelve hours, analytical data for samples at four different temperatures are ready to report. The iChemExplorer with the HPLC helps to automate sampling, analysis and temperature recording in a single workstation for better quality data.

Preparation of Chemstation online with Checklist for Analytical Method:

- Sign-in to the PC desktop; this reinitiates the Agilent Chemstation application. Any prior session running with a previous user is lost. Best to check what's on the desktop before proceeding
- Open Chemstation and select On button on system diagram window; All modules should appear green as prepared to run.
- From top bar select Method>Load Method to open dialog to select the method with which the samples are to be analyzed.
- Select Edit Entire Method to confirm use of analytical method with iChemExplorer according to checklist below:
 - Pump window – Check mobile phase selections to source bottles; Set Stop Time to pump table run time. The pump run time should Stop Time for all following modules.
 - Autosampler - Select Injection with Needle Wash and set vial position as 71. Select More>> to extend window. Set Draw Speed at 10 ul/min and Draw Height as 10 mm (2 mm default plus 10 mm from bottom of vial to correspond with 1 ml fill mark on vial.
 - Column – Note the column temperature and valve setting
 - Detector – confirm selection of wavelengths
 - Signal Detail – From the Available Signals list select the wavelengths and Add to Method to include on the Signal Description list below.
 - On the Edit Integration Events window select OK to skip
 - Specify Report window – Confirm File is selected with File Types .CSV (worksheet with integrated peak data by wavelength) and .EMF (bit map of chromatograph with integrated peaks). The filename for these files concatenates the File Prefix entry with two digits followed by the filetype.
 - Select OK for all remaining windows to skip to the last window.

- Run Time Checklist - Confirm selections for Data Acquisition and Standard Data Analysis for the data to be acquired and analyzed as the sequence is run.
- Select OK to close.

You may wish to save the method with changes by a new name. Go to Method>Save Method As to open a window to save the method. Consider saving to a unique name (such as preceded by "i") to signify that the method is compatible iChemExplorer.

Solubility Tool for Sequence Table and Heat Table

iChemExplorer works with Agilent Chemstation to coordinate sampling with temperature control. Chemstation continues to drive the HPLC to run methods according to the Sequence Table. And iChemExplorer controls ICEtray temperature. With the Solubility Tool, iChemExplorer creates the Sequence Table in Chemstation with a coordinated Heat Table in iHeat to automate the experimental set up.

- Open the iChemExplorer application such as by selecting the iChem logo on the PC Desktop.
- The iChem software opens to the main page.
 - Across the top are selections starting in the left corner with File, then Edit and so on.
 - On the left side is a graphic of the vial tray. The default layout is the ICEtray for 2 ml vials.
 - To the right of this graphic is a window headed by three tabs –
 - iGraph to view and edit data in the window
 - iHeat to set control of tray temperature
 - iSample to create a sample sequence in Agilent Chemstation
- Select the iSample tab. Entries here are transferred directly to Chemstation. Agilent Chemstation on-line should be running in the background. The order to navigate the entries in iSample is important. Take a moment to find the following fields on the iSample tab – Sampling Method, Sampling Injection Volume and the Active R.V. list.
 - At the Sampling Method drop down list select the analytical method. This list is populated from the Method Folder in Chemstation and should appear in the Sampling Method in the Sequence Table.
 - In the Sampling Injection Volume enter a value from 0.1 ul to the sample loop volume. This value is entered to the Injection Volume on the Sequence Table to override the volume specified in the Method.
 - Select the vials positions on the Active R.V list. Sample vials should be loaded to positions 41 to 49. Select a position greater than the last sample vial for a blank injection such as position 61.

- Select Edit Sequence Parameters to open the Sequence Parameters window in Agilent Chemstation
 - In the Data File Section, confirm that Auto is selected. This is required for use with iChemExplorer. This selection sets the naming of the folders that hold sample data. The folder name is XXX-XXXX where the first three positions indicate vial location, the second two positions are for order in the sample sequence and the last two are for number of injections per sample.
 - See the Path and Subdirectory for the location of data to acquire into iChemExplorer for viewing and export to Microsoft Excel..
 - Select OK to close.
- On the iSample tab, select Solubility Tool. A window opens to enter four set point temperatures and equilibration time to hold at each set point.
 - Enter four temperatures at which to sample. All four entries must be filled.
 - Set Equilibration Time from the drop down list for number of hours.
 - Select Create Temp and Sampling Sequence.
- A status window will open with a message that the Temperature Control and Sequence Table are complete.
- Close the Solubility Tool window and status window will close as well.

Confirmation of Heat Table in iHeat and the Sequence Table in Chemstation

- On the iChemExplorer main page, select the iHeat tab to view the Temperature Control Table. The fields for Elapsed Time and Temperature should be populated with lists that show elapsed times that equilibration time plus sample run time with the corresponding set point in ascending order.
- Go to Agilent Chemstation on-line to check that the Sequence Table has been created. The first indication is that the tray locations selected on the Active R.V. list are populated on the tray graphic in Chemstation.
- Open the Sequence Table. This table follows a template from iChemExplorer. The Template starts with a sample from the lowest vial location in the Active R V list. Then a waitsol method is inserted before the next sampling method. This waitsol method is 60 minutes long. It starts with an injection from a blank vial followed by a turn down in pump rate to 0.1 ml/min. The pump rate is turned up to 1 ml/min by the end of the method. The number of injections to repeat the method should be the same as the number of hours selected for equilibration time. Sampling of the solution vials should follow with a last sample from the blank. The cycle of equilibration followed by sampling is repeated for the three remaining set points.
- Some work is required to make the Sequence Table appropriate for use. See the following items on the Sequence Table
 - Confirm that the Sampling Method as selected in iSample has been populated to the Method Name in the Sequence Table.

- See that the number of injections for the WAITSOL method matches the number of hours selected for equilibration time on the Solubility Tool window.
 - On the Sequence Table, change the Vial location for the WAITSOL method from 1 to 21. Do this for all WAITSOL method entries in the Sequence Table. Place a blank vial in position 21 on the ICEtray.
- Start temperature control first.
 - Go to the iChemExplorer main page and select the iHeat tab. See that the Heat Table is populated with Times and Temperatures.
 - Select Start Ramp. In the Elapsed Time Section see that the Time field is populated. Then the temperature from the ICEbox display should appear in the Temp field in the Instrument Actuals section. Eventually the active entries in the Heat Table will be highlighted in blue. The ICEtray is under control and temperature is being recorded.
- Start the Sequence Table after iHeat is underway. Select Start Sequence on the iSample tab in iChemExplorer. Or start and stop in Chemstation the usual way.

iChemExplorer should remain open and running as Chemstation executes the Sequence Table. Over this time, iChemExplorer communicates with the ICEbox to maintain the set point and record ICEtray temperature. iChemExplorer also scans the data file location for new folders. iChemExplorer records the ICEtray temperature to the new folder to capture the temperature at the time of sample. This record is critical to the quality of the data for further analysis.

Notes:

Sample Preparation

- Prepare 10 ml of each solvent with solute. Solution should be supersaturated – undissolved solute visible in solvent at ambient temperature.
- Recommended materials for sample vials
 - 2 ml crimp top vial
 - 11 mm crimp top
 - White silicone/red PTFE septum
 - 2 x 5-7 mm magnetic stir bar
- Fill sample vials to 1.5 ml
- Prepare two blank vials for the Sequence Table from the Solubility Tool.

Method Development

- The greatest concern in preparing an analytical method for use with iChemExplorer is confirming that the signal for the peak of interest is within range of the detector. Often, the method is developed to maximize sensitivity for the peak of interest from samples that have been diluted. With the iChemExplorer, samples are drawn at the full strength of the solution.
- Consider running some calibration solutions; at 10 mg/ml and another at 80 mg/ml. See that the signal response is within range of the detector. The calibration data will prove useful for conversion of experimental data to mg/ml.
- Reduce the injection volume. Sample volumes to 0.1 ml are repeatable with the Agilent autosamplers.
- Consider an alternative wavelength with linear response over the range of interest. Perhaps less sensitive, the signal is within the range of the detector over the sample run. With the diode array detector, additional wavelengths may be recorded with no effect on the method run time.

Experiment Run Time

- The total experiment time includes the run time for the initial sample and then the cycle of equilibration time and sample run time for each set point temperature. Consider that the equilibration time. For an equilibration time of two hours and a run time of ten minutes for four solution vials, the total experiment run time is 13.5 hours. The total solvent consumed is less than 1000 ml.