Use of iChemExplorer and Waters Empower

To Create Time Temperature Studies

With the Agilent HPLC

For use with iChemExplorer version 8.0.1 and higher.
Please visit www.ichemexplorer.com to upgrade to latest version.

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iChemExplorer

Introduction

The iChemExplorer hardware brings heating and stirring to the Agilent HPLC autosampler to move chemistry from the hood to the sample vial. iChemExplorer controls and records temperature as the autosampler take samples. Then iChemExplorer acquires the data to create peak profiles – lines that follow peak area from sample to sample – so you can watch you chemistry as it happens. The iChemExplorer exports the peak profiles, chromatographs and data in numeric format to Microsoft Excel. As an Excel workbook, the results are ready for presentation and storage to an appropriate medium such as electronic lab notebook.

Notes to Get Started

- Continue to use Waters Empower to control HPLC operation – to write methods, to create a sampling sequence, and to integrate peak area.
- Use the Export Method Editor in Waters Empower to create a local data file with integrated peak area as each analytical method in the sequence is complete. The data file storage location must be accessible to the iChemExplorer software as the sequence is running.
- The ICEtray replaces the Agilent HPLC 100-position tray for 2 ml vials. The blocks holds 57 2 ml vials in the same orientation in a single heating zone from ambient (22 C default) to 150 C.
- The ICEbox goes under the Agilent HPLC autosampler to provide magnetic stirring to vials in the ICEtray up to 1000 RPM.
- Agilent HPLC performance specifications remain unchanged with installation of iChemExplorer hardware and interaction with iChemExplorer software.
- iChemExplorer can operate on a PC separate from Waters Empower. The PC must have read-write access to the storage location for the local data files from sample sequence.
- iChemExplorer is compatible with Microsoft Windows 8.1, 7 and XP.
- iChemExplorer does require access to Microsoft Excel. iChemExplorer is compatible with all versions of Excel from Office 2003 to Office 2013.
- As iChemExplorer "talks to" the ICEbox by RS-232, the PC must have a serial port – 9 pin D-Shell male connector. A USB to Serial Port Adapter does not work. An Ethernet to Serial Port Adapter can work. Distance from the PC to the ICEbox should be less than 3 meters.

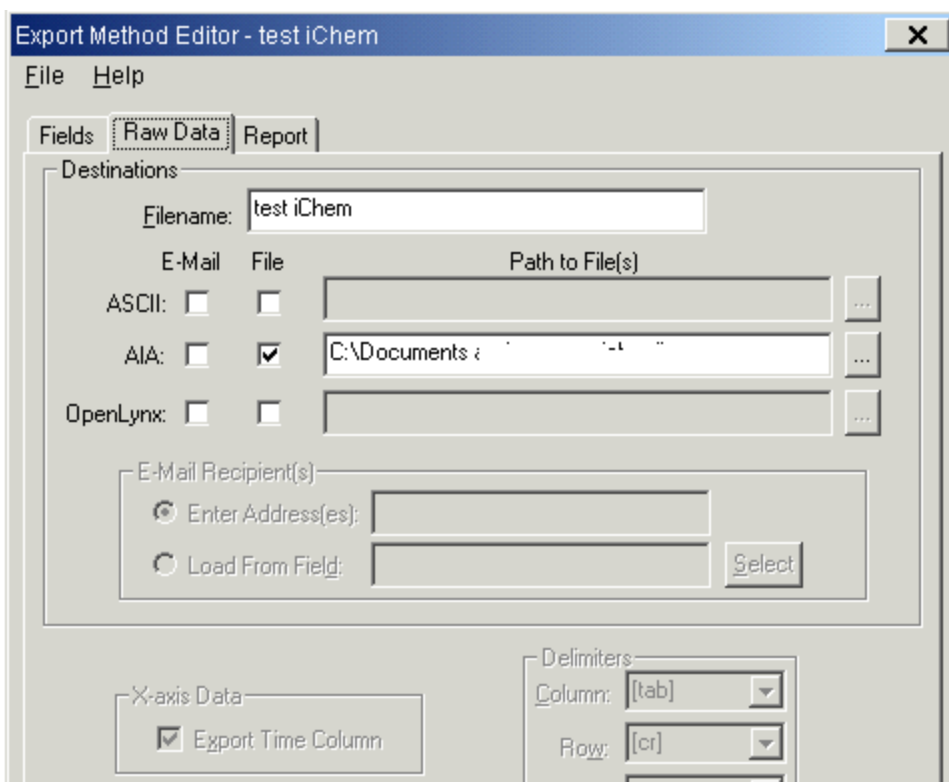## RECOMMENDATION: Verify Analytical Method is Fit for Purpose

An analytical method developed for single point sampling may need adjustment to be suitable for a time-temperature study. Check that the UV absorbance by the material of interest is within the range of the detector over the expected change in concentration for the study. One way to resolve this is by reducing the injection volume. The Agilent HPLC autosampler provides reproducible volumes to 1.0 micro liter. Also consider an alternate wavelength that may be less sensitive yet still provide a linear response over the concentration of interest. Add needle washes to the method to minimize carryover. A robust method makes a robust sampling sequence.

iChemExplorer

Create an Export Method in Waters Empower

Open the Empower application. Select the Browse Project icon to open the Project window. From the top bar select File>New>Export Method to open the Export Method Editor window as below.

In the Export Method Editor window, select the tab for Raw Data.
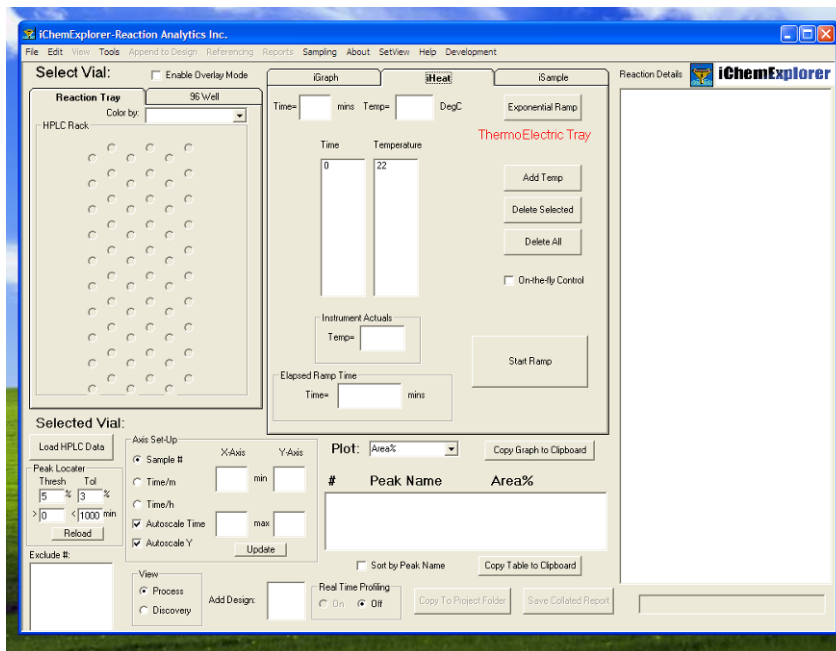

In Destinations, enter the file name for the local output file in the Filename: field. For AIA select File and for Path to File enter or browse to a location accessible to the PC with iChemExplorer. Select File at the top of the window to name and save the Export Method. This method will be available when specifying the Sample Sequence Method Set in the drop down list for Export Method.
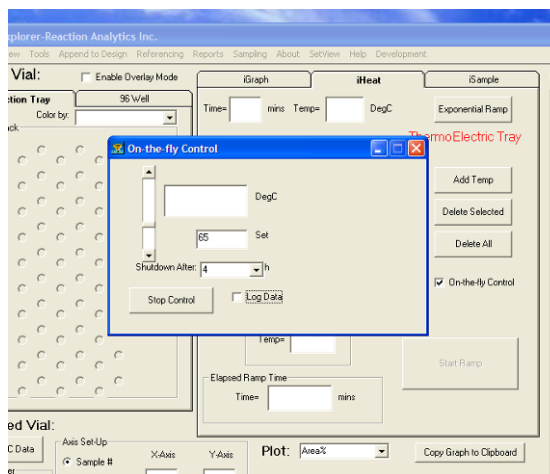
The AIA file selection results in a file with file type .CDF for common data format. iChemExplorer is able to parse the integrated peak area as well as a bitmap of the chromatograph from this data. The filename includes the text as entered in the Export Method with a sequential number generated by Waters Empower.

![iChemExplorer logo]

Procedure to Set ICEtray Temperature Profile in iChemExplorer iHeat tab
Select the iHeat tab from the iChemExplorer main page for three tools to control
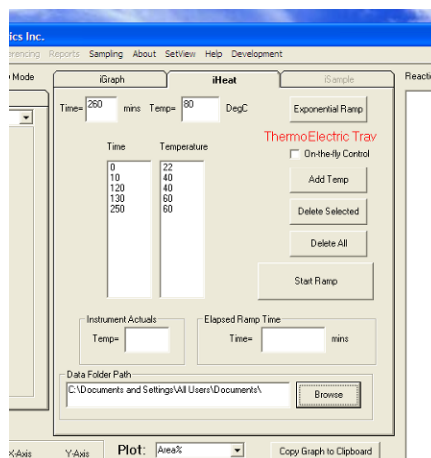ICEtray temperature.

- **On the fly Control** – Select to open a window for "manual control" of ICEtray
  temperature. Use the slider and up and down arrows to select the set point
  temperature in the SET field from 22 to 150 Celsius.

Select the Shutdown Time in the drop down or enter the desired value in
Hours. Select Start Control for iChemExplorer to start temperature control of
the ICEtray. Select Log Data to open Windows Explorer to set a file name and
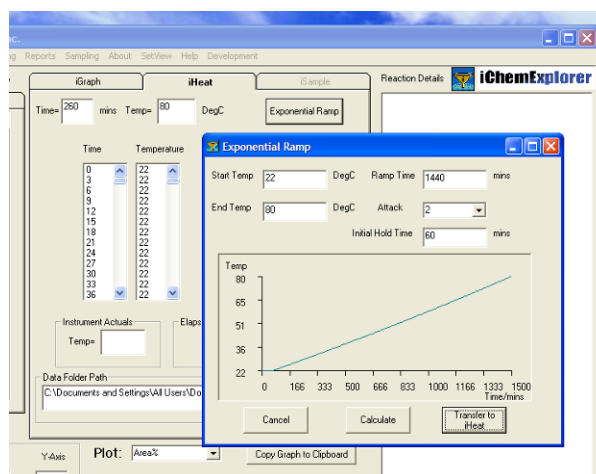select a storage location.

- **Heat Table** – In the center of the iHeat tab see two columns – (Elapsed) Time and Temperature. The columns start with defaults – 0 minutes and 22 Celsius.

Enter values for elapsed time and set point in the Time and Temp fields. Select Add Temp to bring these values into the table. For changes to the table, select the row and select Delete Selected. Select Delete All to remove all entries to the table. Use the Heat Table to make temperature steps – ramp to set point and then hold to reach steady-state and then sample. Ramp to the next temperature and repeat...

- **Exponential Ramp** – Select to open a window to make temperature ramps.
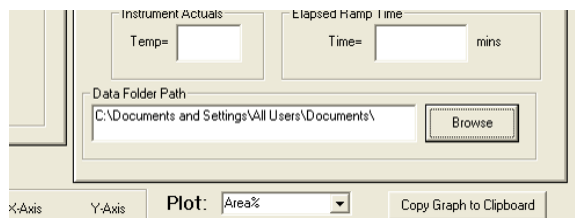

Enter the Start and End Temperatures. Enter the Ramp Rate or change the value for Attack from 1 to change Ramp Rate to Ramp Time and enter the total time in minutes for the ramp. Initial Hold Time refers to a period before the ramp starts. This lead time is used for sampling before the temperature ramp affects chemistry. Select Calculate to display Set Point over Time in the

window. Select Transfer to iHeat to populate the Heat Table with the appropriate set points at three minute intervals to achieve the ramp.

Select Directory Location to Store Templog and to Monitor for New Data Files
With iChemExplorer all the recordkeeping is automated with all the data in a single location. See the bottom of the iHeat tab to find the field to enter the Data Folder Path. This is the location to which the local analytical data files are loaded.

Copy and paste the location from the Output Method Editor file storage. Or use the Browse button to set the location with Windows Explorer. When this field is left blank then iChemExplorer records temperature and monitors for new data files in the location from the which the last HPLC data was loaded.

iChemExplorer logs the temperature over the heat ramp in this location. Be careful that the data file location is clear of previous TempLog.CSV file or else the new temperature ramp data will be appended to the existing file. TempLog.CSV may be viewed in Microsoft Excel.

iChemExplorer also scans this location for new local data files. Remember that the HPLC Control Software adds a data file to this location upon the completion of each analytical method in the sample sequence. When a new file is detected, iChemExplorer records the ICEtray temperature to a record with the same name but by file type .TRC. This tile type is unique to iChemExplorer. To view, change the file type to .CSV. View in Microsoft Excel. To use with iChemExplorer return the file type to .TRC. Below are descriptions of the files created by iChemExplorer through a temperature ramp.

[Date Stamp]TempLog.CSV – This log is a record of time, set point and ICEtray that is created each time Start Ramp is selected in iHeat. The location for this file is set on the Data Logging tab in Preferences under File on the top bar of the main page

The frequency with which entries are recorded to TempLog.CSV and to [Date Stamp]TempLog.CSV is set on the Data Logging tab as well. Select a frequency that keeps the log files fewer than 300 entries for the total time of the temperature ramp as this may be practical limit set by the PC.

Select Auto Enable to Load Data Files to iGraph as Sequence is Running
To automate the acquisition of .CDF files go to File in the top bar of the iChemExplorer main page. Select Preferences from the drop down list to open a

window with tabs. On the Miscellaneous tab select AutoScan Enabled to make this acquisition automatic when running a Temperature Ramp with iHeat. As with Real Time Profiling, the periodic scan is indicated on the iChemExplorer main page in a bar on the bottom right corner

NOTE: Start Temperature Ramp after starting the Sequence in Waters Empower
Select Start Ramp on the iHeat tab to start the temperature ramp as set out on the Heat Table. The ramp should be started after the sample sequence is started in Waters Empower. This ensures that iChemExplorer is prepared to record temperature for the first sample in the sequence.

Once the ramp is started see that the fields for Instrument Actuals and Elapsed Ramp Time are populated. This shows that the iChemExplorer software and hardware are communicating as expected.
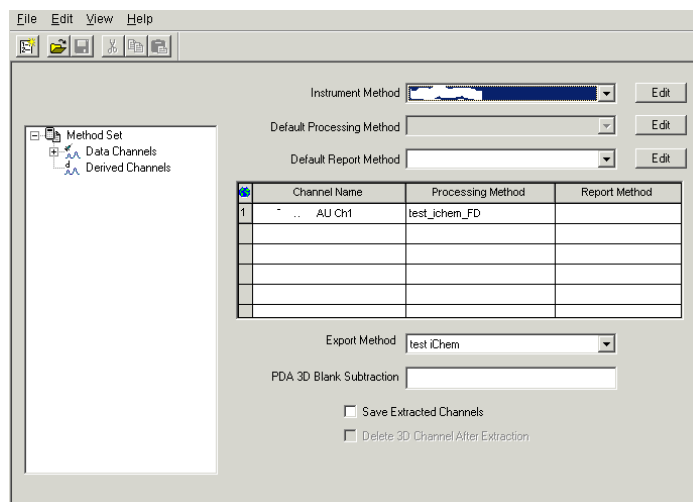
NOTE: Setview to minimize iChemExplorer window to iHeat tab
It may be more convenient to minimize the iChemExplorer window on the PC desktop to the iHeat tab while the temperature ramp and sample sequence are underway. On the top bar of the iChemExplorer main page, select SetView. From the drop-down list select iHeat View. The iChemExplorer window shows the iHeat tab only. This view may then be set on another application such as the HPLC Control Software.
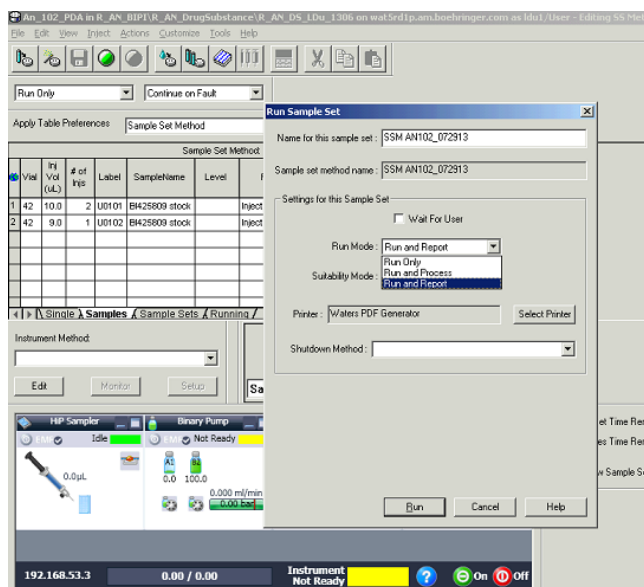
iChemExplorer

Running the Sample Sequence in Waters Empower
Go to the Sample Sequence Set window to select the methods for the Sample Set. From the drops downs for each, select the instrument method, processing method and export method.

Select File and Save.

From the drop-down in the top bar select Run and Report.

With the selection of processing method and Run and Report, Empower should export a data file for each sample in the sequence.
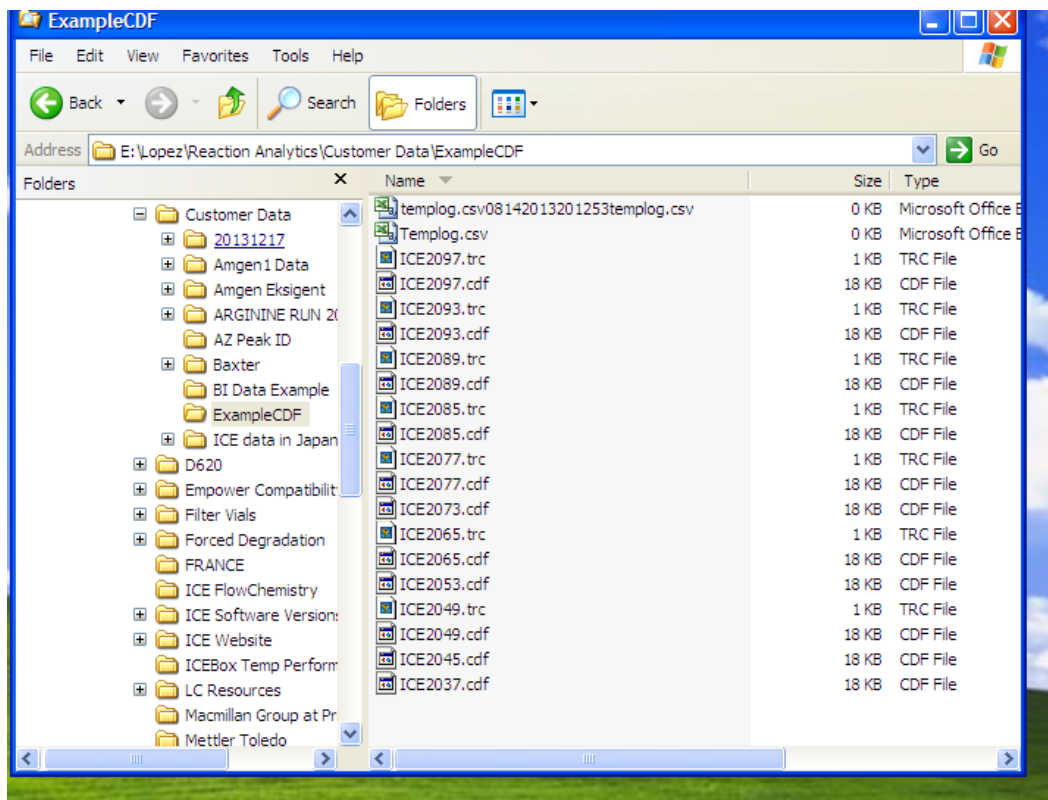
NOTE: The choice of Processing Method may indicate that no integration is to be done at the time of the run. Regardless, a data file is exported to the network

location as each sample is analyzed through the HPLC. iChemExplorer uses the presence of these data files to record temperature. The data may be processed at a later time. The file names for the processed data are the same as for the original files so the temperature records remain associated with the data.

Check that the local data files are created
Use Windows Explorer to view the directory location set in the sequence table to store the local data files. See that the data files appear by the filename with .CDF file type.
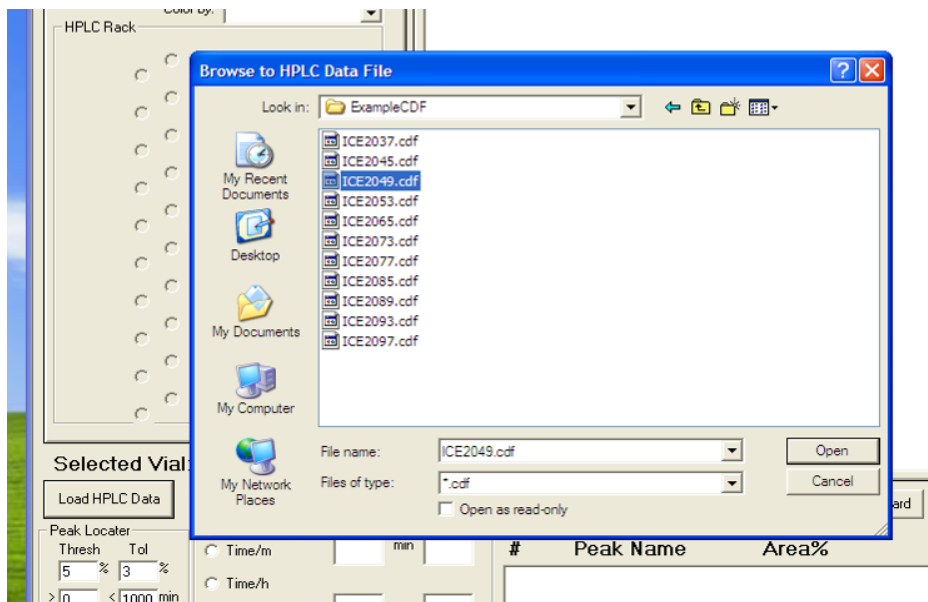

iChemExplorer scans this directory location as set on the iHeat tab. As new data files are added, iChemExplorer adds a corresponding temperature record by the same file name with the .TRC filetype. This file type is unique to iChemExplorer.

In this same directory location also find the TempLog.CSV file. This is a log of temperature over time for the ICEtray. This file is exported to Microsoft Excel in the Collated Report.

iChemExplorer

Load and View data in the iGraph Window

To view the data in iChemExplorer, select Load HPLC Data on the main page. Windows Explorer opens to find and select data to acquire. Remember that with acquisition by iChemExplorer, the source data remains intact. The Explorer window default filetype is .CDF.

Select any CDF file in the directory and select Open. iChemExplorer automatically acquires all CDF files in the directory for display.

Let us know these instructions were helpful at administrator@ichemexplorer.com

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