Agilent OpenLAB CDS ChemStation Edition

Concepts and Workflows

Agilent Technologies

Notices

This product may be used as a component of an in vitro diagnostic system if the system is registered with the appropriate authorities and complies with the relevant regulations. Otherwise, it is intended only for general laboratory use.

Software Revision

This guide is valid for revision C.01.05 of Agilent OpenLAB CDS ChemStation Edition.

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Warranty

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide...

This guide describes the concepts of Agilent OpenLAB CDS ChemStation Edition. In the following, the term ChemStation always refers to Agilent OpenLAB CDS ChemStation Edition.

In this manual we describe the efficient use of the data acquisition, analysis, and reporting functions in OpenLAB CDS ChemStation Edition C.01.05 to boost your lab's productivity.

1 Basic Concepts of OpenLAB CDS ChemStation Edition

This chapter explains the principles of working with ChemStation, including remote control, the graphical interface and the ChemStation views.

2 Working with Methods

The method is a vital part of ChemStation, and this chapter explains the concepts in detail.

3 Data Acquisition

This chapter contains an introduction to the process of acquiring data.

4 Automation/Sequences

This chapter describes the concepts of automation. It explains how to work with sequences in ChemStation, what happens when a sequence is run and how to customize sequences.

5 Run Queue and Queue Planner

This chapter explains the concepts of Run Queue and Queue Planner. It explains how to add single samples, sequences, or pauses to the Run Queue.

6 Data Analysis and Review Concepts

This chapter outlines the data analysis and data review options. In OpenLAB CDS ChemStation Edition, these options are available in two separate views.

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Basic Concepts of OpenLAB CDS ChemStation Edition

This chapter explains the principles of working with ChemStation, including remote control, the graphical interface and the ChemStation views.

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Terms and Abbreviations

Table 1   Terms and Abbreviations used in this document

Term	Description
ChemStation	OpenLAB CDS ChemStation Edition
EZChrom	OpenLAB CDS EZChrom Edition
Data Store	OpenLAB Data Store
ECM	OpenLAB Enterprise Content Manager
RC .Net	RapidControl .Net Interface

Introduction

Agilent OpenLAB is a portfolio of laboratory software, which provides an open architecture and reusable, standardized interfaces. There are different OpenLAB solutions for each step in the life cycle of scientific data:

- Chromatographic Data System (CDS)

  OpenLAB CDS is available as EZChrom Edition or as ChemStation Edition. This manual describes the ChemStation Edition.

- Enterprise Content Manager (ECM)

- Electronic Lab Notebook (ELN)

OpenLAB CDS provides full instrument control of Agilent's LC, GC, CE, CE-MS and LC-MSD instrumentation. It offers tools for data acquisition, analysis and interpretation using a multi-technique, multi-vendor instrument control. You start the chromatography software from the OpenLAB Control Panel, where you access all functions provided by OpenLAB Shared Services.

Remote Instrument Control

With a distributed system configuration, you can configure and launch your ChemStation instruments from any OpenLAB Control Panel that is connected to the OpenLAB Shared Services server.

Launch Instruments

To configure or launch your instruments, you can use the *Configure Instrument*, *Launch online*, and *Launch offline* buttons in the OpenLAB Control Panel. As in workstation or networked workstation configurations, the instrument configuration dialog runs on the local PC. However, with a distributed system configuration, the ChemStation application itself runs on an Agilent Instrument Control (AIC) machine, and you access the application via a Remote Desktop connection to the AIC machine.

The remote ChemStation windows are shown independently of the OpenLab Control Panel; you can launch an instrument, close the Control Panel, and continue working with the instrument. Also you can run multiple instances of OpenLAB Control Panel on the same client using different logon credentials. The different credentials will be propagated to the instruments you launch from the corresponding OpenLAB Control Panel.

You can identify the instruments running on a remote AIC machine by the window title, which contains both the instrument name and the AIC name.

Session Disconnect

Instruments running on an AIC are independent of the client from which you opened the Remote Desktop connection. If the client disconnects, for example, due to network failure, a sequence running on the instrument continues unaffected. To regain control over the instrument once the network has recovered, simply click the *Launch online* or *Launch offline* button again.

To disconnect intentionally, click on the **Close** button or select **File > Exit**. The **Close** dialog offers an additional **Disconnect** button. By disconnecting, you cut off the Remote Desktop connection while leaving the instrument running.

NOTE You can disconnect the Remote Desktop connection while a sequence is running.

To reconnect to this instrument, simply click the *Launch online* or *Launch offline* button again in the OpenLab Control Panel. You can reconnect from any OpenLAB Control Panel that is connected to the OpenLAB Shared Services server.

If you click *Launch offline* to reconnect to an online instrument, or vice versa, you will get two instrument windows, one for the online instrument and one for the offline instrument.

Session Takeover

You can take over an existing session by clicking the *Launch online* or *Launch offline* button in the OpenLAB Control Panel on a different PC:

• If you launched an instrument from the OpenLAB Control Panel on PC 1, then log on to an OpenLAB Control Panel on PC 2 with the same user credentials and launch the same instrument there, you simply take over your existing session and continue on PC 2 with whatever you started on PC 1.

NOTE No warning is shown if the new user and former user have the same credentials.

• If another user has launched the instrument from the OpenLAB Control Panel on a different PC, and you have the required privileges, you can also take over that session. You must have the **Take over ChemStation Remote Session** privilege, and if the other user has privately locked the ChemStation, you must also have the **Break Session Lock** privilege.

If you take over the session, the other user will get a message that you are going to take over the session. As soon as the other user has acknowledged this message, the instrument window is closed on this user's PC and opened on your PC. The other user receives a message stating which user has taken over the session.

Online and offline instruments are included in the same session and are therefore always transferred together. If an online instrument and an offline instrument are already launched in a session, the takeover transfers control of both instruments, regardless of whether you clicked the *Launch online* or *Launch offline* button. If you click *Launch offline* and the session comprises only an online instrument, or vice versa, you will get two instrument windows, one for the online instrument and one for the offline instrument.

About the ChemStation Software

Operating System

ChemStation C.01.05 requires Microsoft Windows XP Professional SP3, Windows Vista Business SP2 or Windows 7 operating system.

The ChemStation Control Charts feature requires Microsoft Excel.

Central Data Storage

A central data storage system can hold all kinds of electronic data independent of any proprietary data formats. ChemStation raw data (and other human-readable documents, such as workbooks) are stored together with *metadata*; this allows the data to be searched very easily. ChemStation methods, sequence templates, report templates, and data files (sequences and single runs) can be uploaded to the central repository, and later downloaded back into the ChemStation if required.

Agilent provides two systems for central data storage:

- *OpenLAB Data Store*, which is available as an all-in-one installation together with OpenLAB CDS. It is designed for small laboratories with up to 15 instruments.

- *OpenLAB ECM*, which is available as a standalone product. It is designed for laboratories with more than 15 instruments.

For more information on the concepts of ChemStation with central data storage, refer to the *Agilent OpenLAB CDS ChemStation Edition with Central Data Storage Concepts Guide*.

Methods and Sequences

The analytical method fully describes how a particular separation is performed. It contains all parameters for instrument control, data acquisition and evaluation, including integration, quantification and reporting. The system may be set up to acquire data from a number of samples by different methods. The control file for this sort of operation is called a sequence, and holds the individual sample information, references to the appropriate methods, and automatic recalibration specifications. For further information on methods and sequences, see "Automation/Sequences" on page 71 and the online help system.

System Configuration

The configuration of the instrument system is done through the OpenLAB Control Panel which starts the configuration editor program. It allows you to define your instruments, their GPIB or LAN addresses, the directories for your data, sequences and methods and the initial screen size of the ChemStation software. In addition, you can activate or deactivate Intelligent Reporting and 3D Spectral Evaluation, and you can define the method download options.

Acquisition Method Viewer

With the Acquisition Method Viewer, you can check the acquisition parameters stored in a method, independently of the current instrument configuration. You can choose to either apply this method in its original version to the instrument, or to resolve this method against the current instrument configuration.

Method Download Options

The method download options define the behavior of ChemStation, if the last selected method from the previous instrument session differs from the current instrument settings. You can choose among the following options:

- **Download method to instrument**

  The last selected method is downloaded to instrument. The instrument settings will be overwritten. This behavior corresponds to ChemStation revisions C.01.03 or lower.

- **Upload method from instrument**

  The instrument settings are uploaded into the last selected method. The method will be marked as modified.

- **New method from instrument**

  The instrument settings are uploaded into a newly created ChemStation method.

- **Always ask user to choose an option**

  At ChemStation startup, a dialog is shown where you can choose on of the options described above. In this dialog, you can also compare the instrument settings for each module with the settings in the last selected method.

  When comparing the differences, you can either display the entire list of settings, or display only the differences.

NOTE   This dialog evaluates the settings of instruments with RC.Net drivers only. It does not evaluate settings of instruments with classic drivers.

Data Model

The ChemStation software is designed around a data model based on a memory structure called a register. Registers are multipurpose structures that can hold analytical data and information for both two-dimensional information (for example, time/intensity) and three-dimensional information (for example, time/intensity/wavelength).

ChemStation provides commands and functions to construct, expand, extract and, where it does not alter primary data, edit registers. For further information, see the online reference in ChemStation under **Help > Commands**.

File Naming Conventions

File Names and Tokens

In most of the ChemStation dialogs where you enter a path name or file name, you can use tokens to dynamically generate suitable names. Depending on the file or path name that is set in a given dialog, different tokens are available. In the following screens, several tokens are used as examples.

The control for file names has the following appearance:

Choose token

Delete content

`<SampleName> <Date> <Time>` ✕ ▶

Tokens are display in blue color and with an underline
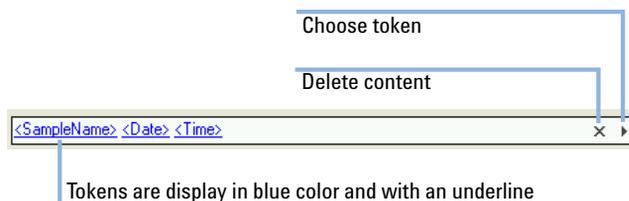
The control for path names has the following appearance:

Choose token

Browse for location

Delete content

`/<Computer>/<InstName>/` ✕ ··· ▶

Tokens are displayed in blue color and with an underline
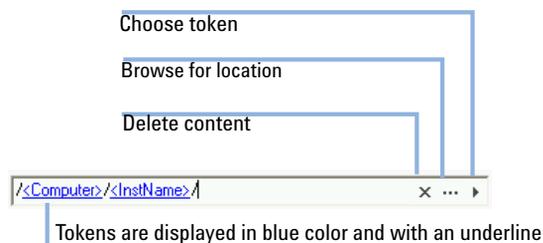
In each corresponding dialog, the resulting file name or path name is displayed additionally.

You have the following options to work with this type of fields:

· Add static text.

· Click the Arrow button (▶) to choose a token from the list.

   Press the **Arrow Down** key to choose a token from the list.

· Right-click one of the tokens already used to replace it by another token from the list.

· Click the X button to delete the current content of the field.

· Click the button with the three dots (⋯) to browse for the required path.

Naming Conventions

The following rules enable ChemStation to create and process valid names for files and directories:

The following characters are not allowed as part of a file or directory name:

< > : " / \ | @ % * ? '

Using these characters in file or directory names may cause problems when loading files in ChemStation. In addition, if these characters are used in the installation folder, the reprocessing copy does not start. If the character % is used in the installation folder, some ChemStation shortcuts do not work properly.

The following rules apply in addition:

Table 2   Restricted characters

ChemStation parameter	Character
Method file names:	% and . (decimal point) are not allowed
Data subdirectory and sequence subdirectory:	[] + = ; , . (decimal point); spaces are not allowed
Data file names in sequences	blanks are not allowed

The following reserved device names cannot be used as the name of a file:

- CON, PRN, AUX, NUL
- COMx (where x is a number from 1 to 9)
- LPT1x (where x is a number from 1 to 9)

Also avoid these names followed by an extension (e.g. Nul.txt).

NOTE English, Japanese, and Chinese operating systems are used to test naming conventions. Agilent cannot give a support statement for non-English operating systems and their special characters.

Maximum Length of ChemStation file names and subdirectories

The Agilent ChemStation specifications for file names and subdirectories are listed below:

Table 3   Maximum length of ChemStation file names and subdirectories

DataFile/Subdirectory/Path	Max. Input Length	Auto append	Example
Data file name of single sample	60	.D	Demodad.d
Data file name in a sequence, using prefix/counter	15	.D	longname000001.d
Method Sequence Hypersequence Libraries Customized Report Templates	40	. M . S . HYP . UVL . FRP	def_lc.m def_lc.s def_lc.hyp demodad.uvl areapct.frp
Data file subdirectory	40		demo (in sample information)
Data sequence subdirectory	40		demo (in sequence parameters)
Result Set Name	40		test_date_time (create using sequence preferences)
Data Path Method Path Sequence Path Hypersequence Path Libraries Path Customized Report Template Path	100	100	c:\chem32\1\data c:\chem32\1\methods c:\chem32\1\sequence c:\chem32\1\hyper c:\chem32\speclib c:\chem32\repstyle

All ChemStation logbooks report system messages in an extended format and information strings are printed over multiple lines. Certain reports, e.g. Sequence report, may truncate filenames to fit all information onto the report template.

Software User Interface

The ChemStation user interface is designed into views that group software functionality according to typical analytical tasks. The following standard views are present in all software configurations:

- the Method and Run Control view for controlling and acquiring data from the instrument
- the Data Analysis view for reevaluating data that has been acquired
- the Review view for reviewing the data using specific report templates
- the Report Layout view for designing specific report layouts

Additional views are present if additional data evaluation modules have been ordered or for certain instrument configurations that support instrument diagnostics and verification procedures. A ChemStation Companion view is available for when it is desirable to have instrument operators run samples from an easy-to-use, preconfigured table.

The Navigation Pane contains the Navigation Button, to allow rapid switching between the ChemStation views, and the tree-based ChemStation Explorer. The contents of the ChemStation Explorer are view-dependent, giving access to different ChemStation elements.

Each view consists of a set of standard user elements including menus and toolbars. The standard toolbar provides rapid access to the common system specification information such as methods and sequences. The Method and Run Control view additionally incorporates a system status bar, a sample information area, which may be configured for single runs or automated runs, and a schematic instrument interface diagram for GC, CE and LC configurations. The schematic instrument interface diagram uses hot spots to allow rapid access to instrument parameters and an animated graphical overview of the status of each analysis as it proceeds. The schematic instrument diagram may be turned off if it is not required, to save memory and other Windows resources.

The Data Analysis view extends the standard toolbar to specific data analysis modes including recalculation, reprocessing, integration, calibration, reporting, annotation, signal comparison and additional specialized modes if the modules are installed. Each of these separate data analysis modes are supported with a mode-specific toolset.

The Review view is available if Intelligent Reporting is selected for the instrument. This view allows you review the data in a very flexible way. You can select any combination of data files as a basis for the review, and apply any existing report template to the selected data. The selected report template defines the way in which the data is shown and which type of information is included in the generated report. The toolbar provides functions for printing and exporting the generated reports.

The Report Layout view allows you to define the layout of a specific report template or report style. It also uses a set of toolbars specific to this task. The type of Report Template editor shown in this view depends on the type of reporting that is configured for the instrument. You can either use Classic Reporting or Intelligent Reporting (see "Reporting" on page 169).

Navigation Pane

A Navigation Pane, available on the left side of all ChemStation views, is designed to speed access to many key ChemStation elements, as well as enabling quick switching between views. The Navigation Pane contains the tree-based ChemStation Explorer and a configurable button area. It also includes an autohide feature so that the ChemStation workspace is not compromised, and offers standard features such as resizing, and re-arranging of the navigation button area.

Navigation Buttons

The Navigation Buttons allow the ChemStation view to be switched by clicking on the specific Navigation Button. The Navigation Button section can be minimized, expanded and re-arranged.

ChemStation Explorer

The contents of the Navigation Pane are view-dependent. For Method and Run Control, Data Analysis, Review, and Report Layout, the ChemStation Explorer allows you to navigate to the different ChemStation elements. By default, these elements for data, methods and sequences are based on the configuration editor settings. New nodes for methods, sequences, data location can be specified using the "Preferences" option in the view menu.

Table 4   Navigation Pane items

Navigation Buttons	ChemStation Explorer Elements
Method and Run Control	Sequence templates / Master methods, Result Set methods
Data Analysis	Data / Master methods, Result Set methods
Review	Data / Report templates
Report Layout	Classic Reporting: Master methods Intelligent Reporting: Report Templates
Verification (LC and LC/MS)	Verification view-specific shortcuts
Diagnosis (LC and LC/MS)	Diagnosis view-specific shortcuts
Tune (LC/MS)	Tune view-specific shortcuts

Data Acquisition

The status of the instrument is continually monitored and updated on the display, along with the elapsed run time of the analysis both when the software is a visible window and when it is iconized. The transactions that occur during the analysis, including any errors and the instrument conditions at the start and the end of the analysis, are recorded in the system's logbook, an extract of which is stored with every data file.

The instrument conditions, such as flow, temperature, pressure and solvent composition for liquid chromatographs may be recorded and stored with each data file. These instrument parameters can be displayed and plotted to testify to the quality of each analysis. The exact nature of the parameters recorded depends both on the technique and the capabilities of the configured instrument.

All regular data acquisitions - single samples as well as sequence runs - are first added to the Run Queue and then started from there. For more information, see "Supported Workflows" on page 126.

One or more display windows may be used to monitor the data being acquired by the instrument in real time. The data are displayed in real measurement units such as mAU, Volts, degrees or bar. The windows may each show multiple overlaid chromatographic/electropherographic signals or instrument

parameters, such as pressure. The display default settings may be adjusted and are remembered by the system so users can set their own preferred settings as the instrument default. The window has zoom capability and the cursor may be used to display a specific signal's response at any point in time.

During an analysis, the complete functionality of ChemStation can be used through the offline copy. While acquisition is running, the Data Analysis part of the online session of an instrument is not accessible, and data review has to be performed in the offline copy.

A snapshot function is available for users who wish to start processing data before the analysis is completed. The snapshot has to be taken in the offline copy of the instrument sessions and is immediately present for review.

The layout of the signal and status information windows, including the components of the schematic instrument interface diagram is saved automatically.

For further information on Data Acquisition, see "Data Acquisition" on page 63 and the online help system.

Data Analysis

Data Analysis — Display

The Data Analysis view extends the standard tool bar with task-grouped data analysis functions including recalculation, reprocessing, integration, calibration, reporting, annotation, and signal comparison toolsets. The following key graphical operations are possible:

- single or multisignal displays selectable when loading the chromatogram/electropherogram,

- overlays of chromatogram/electropherogram from different samples,

- subtraction of one chromatogram/electropherogram from another,

- graphical vertical and horizontal alignment of signals to help visual comparison,

- signal inversion or mirroring to help visual comparison,

- displays of extended peak performance characteristics for specific integrated peaks,

- graphical zoom and scrolling functions,
- adjustment of display attributes including selection of tick marks, baselines, axes, retention/migration times and compound names (you can also select the font for the RT and compound labels, adjust the size and orientation of the display, select the display as overlaid or separated and select scaling factors),
- the chromatogram/electropherogram display may include graphical overlays of instrument parameters depending on the capability of the configured instrument,
- user-defined annotations may be interactively added to the display, with the selection of font, size, text rotation and color (once defined, the annotations may be graphically moved, edited or deleted),
- copy the display to the Windows clipboard in both metafile and bitmap format,
- a *pick mode* function to display the values of individual data points in detector units, and
- export of time/intensity digitized points to the Microsoft Windows clipboard.

Data Analysis — Integration

The ChemStation integrator algorithm is the second revision of a new generation aimed at improved ruggedness, reliability and ease-of-use.

Data Analysis - Quantification

The ChemStation's calibration mode of the data analysis view allows simultaneous display of:

- the signal or signals being calibrated with an indication of the current compound's retention/migration time window,
- the calibration table whose display may be configured from a comprehensive selection of calibration parameters, and
- the calibration curve for the compound being calibrated.

All the calibration mode windows are linked so that changes in one are automatically reflected in all the others. This mode allows graphical selection and modifications of the calibration data.

Quantification is based on %, Normalized %, External standard, External standard %, Internal standard and Internal standard % calculations calculated on either peak area or height. Calibrations may be multilevel and include multiple internal standard definitions. Calibration histories are automatically saved and can be used to weight the recalibration calculations.

For information on calibration and quantitation, see "Calibration" on page 153.

Data Analysis - Batch Review

The following two additional toolsets are available within the Data Analysis view:

- Navigation Table
- Batch Review

The Navigation Table makes possible several key graphical operations:

- standard table configuration features, such as sorting, drag-and-drop options, column selection, item grouping to specify a preferred navigation table configuration
- right mouse click functions to load a signal, overlay a signal, export data, print reports
- review signal details by expanding a line in the navigation table
- review signals and create ChemStation reports using a specific method

The following key graphical operations are possible in the Batch Review:

- define automatic or manual review and reprocess of (calibrated) data files
- recalibration of calibration table
- review compound tables of calibrated methods
- create specific batch reports

Data Analysis - Recalculation

The functions in the recalculation mode allow you to quickly generate results or reports for any subset of data shown in the Navigation Table. You can easily generate results for self-assembled data sets, independent of the sequences the samples were originally acquired in. You can use any method for recalculation. The method used will be copied to the single data files (DA.M). No calibration is done during recalculation.

Data Analysis - Reprocessing

The functions in the reprocessing mode allow you to reprocess one entire sequence, using the methods defined in the sequence table and using the results of the calibration samples to calculate the sample results.

Data Analysis — Last Results

In this mode, the data file method (DA.M) for each run is loaded. DA.M is an exact copy of the method which was used for the last data analysis (during acquisition, reprocessing, or recalculation). The Last Result mode allows you to reproduce the results of the last data analysis, even if the sequence method has been changed in the interim.

Reporting

If Intelligent Reporting has been enabled for an instrument, the **Review** view is enabled, and the Report Template Editor for the Intelligent Reporting is visible in the **Report Layout** view.

With Intelligent Reporting enabled, you can use both Intelligent report templates and Classic report templates to create single injection reports and sequence summary reports. If you have the required privileges you can create report templates for Intelligent Reporting.

With Intelligent Reporting disabled, you can use only Classic report templates to create single injection reports and sequence summary reports. If you have the required privileges you can create report templates for Classic Reporting.

Utilities and Compatibilities

ChemStation can import and export data files in the andi (Analytical Data Interchange) chromatography format of the Analytical Instrument Association (AIA), revision 1.0, copyright 1992. Data import is supported at compliance level one (sample information and signal data) and data export at compliance level two (sample information, signal data and integration results).

ChemStation includes commands and functions to support the dynamic data exchange (DDE) standard of the Microsoft Windows platform as both a DDE client and a DDE server. The command set includes commands to establish and terminate connections, transfer information in both directions and execute remote functions.

Customization

ChemStation can be customized using a powerful command set. These commands may be grouped to execute automatically a specific function; such a group is called a macro. Users writing macros may define their own variables, build in conditional or looping constructs, perform physical I/O including file handling and user interaction, nest their macros and schedule and exchange data with other MS-DOS or Microsoft Windows applications.

For more information on customization, see the online reference in ChemStation under **Help > Commands**.

Automation

ChemStation can plan and execute both single samples and multi-method sequences.

The sequence parameter set may be defined to use automatically generated files or sequentially numbered files with a user-defined prefix of up to fifteen characters. The user may select to run full analyses or data reprocessing only sequences and can also select one of a series of technique specific shutdown commands or a user-defined shutdown macro that runs when the sequence terminates either by error or after all the analyses are completed.

The sequence table, or list of analyses to run, is built in a spreadsheet-like user interface that allows users to specify vial numbers and sample names, sample types, analysis methods, sample quantification parameters including sample amount, a multiplier and dilution factor, calibration specification, data exchange parameter LIMSID and the number of repeat injections. Depending on the configured instruments and modules, more fields will accessible, for example, if an Agilent 1100/1200 LC system includes a fraction collector, the **Fract. Start** column will appear in the sequence table. The appearance of the sequence table can be configured by the user. The user can jump between individual cells in the table and copy, cut or paste individual cells or entire rows or series of rows in order to build sequences efficiently and quickly.

Samples may be identified in the sequence table as unknown, calibration, blank, or control sample types. The sample type determines any special data evaluation treatment of the sample:

- Unknown samples are evaluated and reported according to the method specification.

- Calibration samples are used to recalibrate the quantification component of the method as described below.

- Blank samples are used to evaluate the reference signal for specific peaks, as defined by European Pharmacopeia. You can print the signal-to-noise ratio in customized reports. Please refer to the Reference Guide for details on the calculation and the required data fields.

- Control samples are evaluated against the limits for each component defined in the method. If the results lie outside any specified parameter range the execution of the sequence will be halted.

Calibration samples may be defined as simple, cyclic or bracketed. Simple recalibrations mean a recalibration occurs each time a calibration sample is defined in the sequence. Cyclic recalibrations occur at defined intervals during analysis of a series of unknowns. In bracketing a series of unknown samples, two calibration sets are analyzed. The quantitative reports for the unknown samples are then calculated using a calibration table averaged between the two calibration sets.

The partial sequence functionality allows users to see the order of execution of the sequence and also select individual sample entries to rerun or re-evaluate. When re-evaluating data already acquired users can specify whether reprocessing uses the original sample quantification data or new data entered in the sequence's sample table.

Sequences may be paused to run single injection priority samples by another method, then restarted without disrupting the automation. Samples can be added to the sequence table while the sequence is executing.

Both the sequence and partial sequence tables may be printed.

For more information on sequences, see "Automation/Sequences" on page 71, and the online help system.

Run Queue and Queue Planner

The Run Queue allows you to automatically run several single samples or sequences one after another. The first item added to the queue starts when the data system is ready, unless paused from the queue. You can add single samples, sequences based on Easy Sequence templates, classic ChemStation sequences, or pauses to the queue. Also each **Run Method** or **Run Sequence** command automatically adds an item to the Run Queue, and automatically starts this item in the queue.

Using the Queue Planner, you can prepare a series of single samples or sequences and save the plan to the file system. To start these planned samples and sequences, you simply open the plan and add it to the Run Queue. This function allows you to start lengthy tasks such as overnight or weekend jobs.

For more information, see "Supported Workflows" on page 126.

Good Laboratory Practice

ChemStation is developed to internationally recognized design and development standards and has a number of features specifically to help users operating in a regulated environment. These features are in the area of complete method specification and verification that the methods are fit for their intended use, to check the operation of their system and ensure the traceability, originality and quality of the data.

Development Process

The Certificate of Validation shipped with each software package documents the software development and testing steps executed as part of the development cycle. The development process is registered to the ISO 9001 quality standard.

Method Specification and Use

- Global methods — the complete instrument and data analysis specification is stored in one place. Methods include individual compound range specifications to check that quantification results are not applied outside the calibrated range.

- The method change history log allows users of a validated method to automatically record how and when a method was changed. Users may optionally add a comment reason to the change history log. The change history log is automatically stored as part of the method in a binary format. To prevent unauthorized access to the records, it is protected by the user access scheme, described below. The change history log may be viewed and printed.

- Limits may be assigned on a compound-by-compound basis in each method for a number of chromatographic/electropherographic and system performance parameters, as described in the data analysis quantification section. Results exceeding these parameter ranges are used to control the execution of automated sequences as described in the automation section. They are indicated on the appropriate analysis report.

- System performance or suitability reports (see the Reporting section above) provide detailed analysis of the separation quality.

You can set up different roles and privileges in OpenLAB Shared Services. The pre-configured roles **ChemStation Administrator**, **ChemStation Lab Manager**, **ChemStation Analyst**, and **ChemStation Operator** offer a basis for the roles in your environment.

Method Robustness

Sequence summary reports (see "Classic and Intelligent Reporting" on page 171) provide a means to test methods for robustness. With classic reporting, the extended format reports for user selected criteria are reported as trend charts and may be used to determine the realistic operation limits. With intelligent reporting, you can create your own templates for sequence summary reports including trend charts with limit lines. These limits can then be incorporated in the method to ensure, through the analysis of control samples, that the method is operating within specifications.

System Operation

The ChemStation verification kit, which is part of the standard software, automatically checks for the correct installation and operation of the data evaluation parts of the software by comparing results generated when the test is executed against prerecorded known values. The verification kit allows users to define their own data files and methods to be the basis of the test.

Data Traceability, Originality and Quality

The run time logbook provides a transaction log of the complete system. It also records any unusual events (such as errors or parameter changes made during a run) as well as the instrument conditions before and after each analysis. A copy of the relevant logbook extract is saved with each data file.

The actual instrument conditions, such as pressure, flow, and temperature, that occurred during each analysis are also recorded if the configured instrument supports this capability. This data can be subsequently displayed graphically with the chromatogram/electropherogram to show the actual instrument conditions during that particular analysis, as well as being included on the report.

Methods saved with the data file record the actual method at the time of the analysis and allow the complete reconstruction of the reported data at a later date. The method is saved at the completion of all the analytical steps.

By default, all reports have time stamps and traceable page numbering (*page x of y* pagination style). The user may select the level of detail in each report ranging from simple summary reports to complete system details.

GLP Save register files, specified as part of the method configuration, save all the original data, including sample information, data analysis method, chromatographic/electropherographic signals, instrument conditions, integration and quantification results, report data and the run logbook in one checksum protected binary file. This is an uneditable binary format that ensures the originality of the results. The file includes a revisioning scheme that indicates if data has been reprocessed.

Control sample types may be defined in the sequence table and used to automatically check the instrument performance against quality control sample results when the instrument is running unattended. Results that are outside the user-specified acceptable range will stop the automatic execution of the instrument.

ChemStation Data Structure

Without Unique Folder Creation

This data structure corresponds to the data structure used in ChemStation revisions B.01.03 and earlier. Sequences, methods and the generated data files and results are stored in fixed, specified and separated locations. For example, methods are referenced by name in a sequence and it is the user's responsibility to maintain the integrity of methods, sequences, and data files. Because of this, the long-term archiving of data and reproduction of results is a tedious task. Users have to document the chromatogram, results, and associated method; this is the case not only for regulated labs, but also for some areas of unregulated labs (such as environmental labs). Without result set creation, this can be achieved only by printing everything in a report.

However, there may be situations where users may want to store their data as in ChemStation B.01.03 or earlier and work according to the corresponding workflows:

- During method development it may be more convenient to have only one method for both acquisition and data analysis to automatically have changes available for future acquisition and reanalysis of already acquired data.

- Customized macro solutions on a ChemStation system that have been designed for older revisions may require the data, methods, or sequence to be stored according to the old data organization scheme.

- When ChemStation C.01.05 runs in a lab where there are also systems still running on ChemStation revisions B.01.03 or earlier, it may be more convenient to use the same data organization mode on all systems.

With Unique Folder Creation

In order to strengthen the association between data files and methods, the usage of result sets has been introduced with ChemStation B.02.01 (result sets have been called sequence containers then). If you use a central data storage system (*OpenLAB ECM* or *OpenLAB Data Store*), the complete result set (sequence/methods/data files/report templates) is transferred to the central repository as one entity.
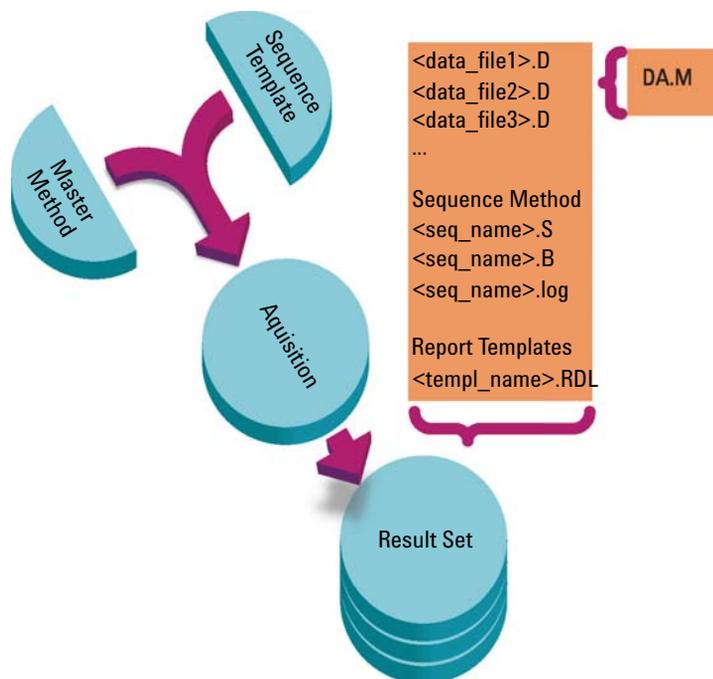
Figure 1   Sequence Acquisition with Unique Folder Creation ON

The methods in the folder Chem32\1\methods serve as master methods. They remain unchanged during acquisition and data analysis.

Similarly, the sequences in the folder Chem32\1\sequence serve as sequence templates that can be used to rerun (but not reprocess) a sequence several times.

Report Templates in the folder Chem32\repstyle serve as a starting point for developing your own report templates.

The data storage pattern varies depending on whether single run data or sequence data is acquired:

1 When a sequence is executed, a new folder is automatically created (**result set**) with a unique name in the specified subdirectory. When a single sample is run, the data file (*.d) is written to the specified subdirectory.

2 For sequence data, the executed sequence template (*.s) and all the methods (*.m) involved are copied into the result set. The copies of the methods are called the **sequence methods** in order to distinguish them from the original master methods. If you use Intelligent Reporting, all involved report templates (*.rdl) are copied into the result set as well.

All sequence-related tasks (for example, acquisition and data analysis) are performed on the copies of the sequence and the methods. Therefore, the sequence template and the master methods remain unchanged for future sequence execution.

Any changes performed on the sequence during sequence acquisition, for example, adding lines to the sequence table, are performed on the copy of the sequence file in the result set. The sequence template remains unchanged.

Similarly, any changes in the method, for example, updates in the calibration table in case of calibration runs, are reflected in the sequence methods, but not in the master methods.

While executing the sequence, all generated data files (*.d) are stored in the sequence data folder, along with the corresponding batch file (*.b) and sequence log file (*.log).

3 Each data file contains a copy of the method used to create the run. The following method information is stored:

- The acquisition parameters are saved as ACQ.TXT to ensure the preservation of the original method parameters for each specific data file. The parameters can be viewed and printed using the **Method > View Method** command.

- The complete method, including data analysis parameters, is saved after completion of the data analysis part as DA.M.

Working with result sets provides a number of advantages:

- Sequence data are not overwritten. Each sequence acquisition stores the resulting data files in its own result set with unique name.

- With the result set concept, the data is stored with all necessary information needed for data analysis: copies of the sequence file, of all methods, and in case of Intelligent Reporting also of the report templates employed with the sequence. The sequence methods can be changed with sequence specific input and do not influence the original master method. The result set concept thus strengthens the meaning of a sequence as a set of data files and methods belonging together for result creation.

- Data recalculation and reprocessing are both available in **Data Analysis** view via the Navigation Table.

- The result set concept provides the optimal preconditions for the data exchange with a central data storage system.

Switching Unique Folder Creation ON or OFF

In order to allow working with a data storage concept as in ChemStation revisions before B.02.01, the **Sequence** tab of the **Preferences** dialog box contains a **Data Storage** section. Here you can choose between **Unique Folder Creation ON** and **Unique Folder Creation OFF** (see "Preferences - Sequence Tab" on page 97). Per default, **Unique Folder Creation ON** is selected. **Unique Folder Creation ON** enables the data storage concept as outlined in the chapter above.

2
Working with Methods

The method is a vital part of ChemStation, and this chapter explains the concepts in detail.

Agilent Technologies

What is a Method?

A method comprises all the parameters for acquisition and data analysis together with pre- and post-run tasks for a given sample, if they are needed.

The available methods (*.m) files are visible in the ChemStation Explorer. For quick and easy navigation, you can add additional method locations to the ChemStation Explorer selection tree using the **Paths** tab of the **Preferences** dialog box.

Parts of a Method

A method is identified by a name of up to forty alphanumeric characters. The file name always has the .M extension to identify it as a method. Methods are stored as directories that contain individual files relating to the components of the method.

Each method comprises four components:

- method information,
- instrument control,
- data analysis, and
- run time checklist.

Method Information

This section is used to define descriptive information about the method.

Instrument Control

Defines parameters that control the instrument or its components. With an LC instrument, parameters, such as mobile phase composition, flow rate, injection volume, detector wavelength, and so on, control the pump, the injector and the detector. With a GC instrument, parameters such as inlet temperature, inlet pressure, packed column flow setting, and so on control the instrument.

Data Analysis

Defines parameters that control the data processing.

- *Signal Details*

  Defines signals and their properties used for data evaluation.

- *Integration Events*

  Defines timed events that will occur at specific retention/migration times on a chromatogram/electropherogram. These timed events can be used to change the way the signal is integrated.

- *Peak Identification*

  Defines data processing parameters associated with the identification of peaks in the chromatogram/electropherogram.

- *Peak Quantification*

  Defines data processing parameters that affect the quantification calculations which determine the amount or concentration of sample component corresponding to each peak.

- *Calibration and Recalibration*

  Defines data processing parameters that affect the calibration and how often calibration is done.

- *Custom Fields*

  Defines the properties of sample or compound related custom fields that are available for the method. The custom fields allow you to add custom information to a sample or a compound in a sample.

- *Report*

  With Classic Reporting: defines the format of the report that is printed after a run.

  With Intelligent Reporting: specifies the report template used to generate the report after a run.

Run Time Checklist

Defines which parts of the method are executed when the method is run.

You can use the run time checklist to:

- acquire, store and process data to produce a report,
- execute only a portion of the method,
- acquire and store data without analyzing it,
- reanalyze existing data files,
- use your own macros for data analysis, pre- and post-run processing, and
- save the analysis results in a register for GLP purposes.

Types of Methods

There are different types of methods. Depending on the storage location, methods are either used as master methods, as a reference within the result set of a sequence, or as an actual record of the settings used during data acquisition.

Master Methods

These are methods stored on the computer disk. Stored methods have a name with up to forty alphanumeric characters followed by the extension *.M. The master method directories are configured in the Preferences (see "Path Selection" on page 64).

The Master Method is stored in a methods subdirectory, available in a Methods node of the ChemStation Explorer, and is not directly associated with any result set.

Sequence Methods

When a sequence is run (using the option **Unique Folder Creation ON**, see "Preferences - Sequence Tab" on page 97), copies of all the master methods used in the sequence are stored in the result set along with the sequence data files. These methods are directly linked to the sequence, and are also used when the sequence is reprocessed. Changes to these methods are by default not automatically propagated to the master methods. Changes take effect as soon as the sequence run starts or continues after a pause. Changes are also propagated to the data file methods (DA.M) when the sequence is reprocessed, and on any report generation.

Data File Methods

A copy of the data analysis parameters is stored as the data file method DA.M with the data files. DA.M is also referred to as the data analysis method.

The data file method DA.M is automatically updated after each result generation (data acquisition, recalculation or report generation). It is also loaded by ChemStation when you recalculate results in the Last Result mode (see "Last Result Mode" on page 139).

If you use the **Save method with Data** option in the run time checklist, the method is stored additionally as run.m in the data file.

In the ChemStation Explorer, you can easily load either a master method or a sequence method by double-clicking on the method item.

Creating Methods

Creating a new method always means modifying a master method or sequence method and saving the modifications. You can either overwrite an existing method, or save a method as a new master method. Be aware that when you change a method, the disk version remains unchanged until you save your changes.

You have a choice of how to create a method. You can create a method to do either one or all parts of an analysis. For example, you can create a method to do only data acquisition. When you are ready to analyze the data and generate a report, you can then modify the method to do these data processing tasks.

NOTE	Do not delete the default method (DEF_LC.M, DEF_CE.M or DEF_GC.M). These method files are used as templates to create new methods.

Editing Methods

You can edit an existing method using the Edit Entire Method item of the
Method menu. You are guided through all the method dialog boxes and at the
end you can save the method. This process is shown below:

Method on disk

Load Method

Method loaded into memory, becomes current method

Select 'Edit Entire Method' from the Method menu

Edit Method

Save Method [As]

Save to new name   Save to same name

Creates new method   Overwrites current method
on disk
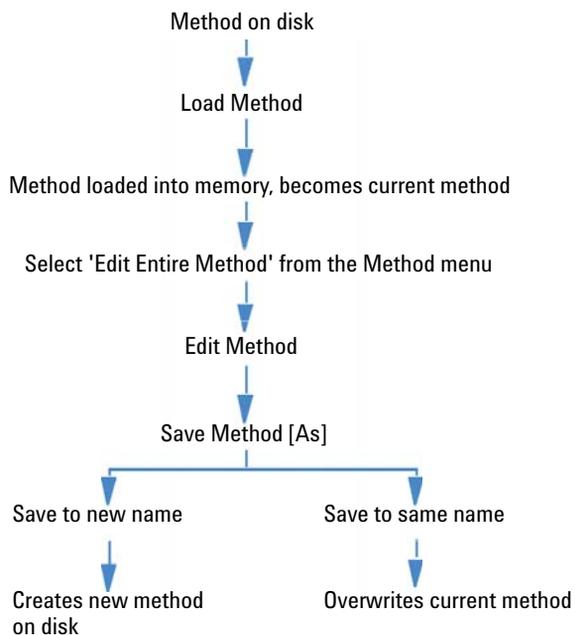
Figure 2   Editing Method

Method Parts to Edit

Each method comprises four components that can be edited separately.

Some of the following subsections refer to specific dialog boxes, and some are generalized descriptions.

- *Method Information* comprises:
  - a text description about the method.
- *Instrument Control* depends on the configuration, and can comprise, for example:
  - oven parameters,
  - injector parameters, and
  - detector parameters.
- *Data Analysis* comprises:
  - signal details,
  - integration parameters,
  - quantification parameters,
  - calibration parameters,
  - custom field setup parameters, and
  - reporting parameters.
- *Run Time Checklist* comprises:
  - the parts of the method that will be executed.

Method Directory Structure

Folders

A method comprises a group of files stored in the method directory (*.M).

By default, master methods are stored under Chem32\1\METHODS. Additional paths for master methods can be added using the preference settings. Sequence methods are stored in the result set, and data file methods are stored as DA.M in the data file subdirectory.

Files

Method files with the .MTH extension contain parameter sets and are in UNICODE format. The file INFO.MTH comprises the method control parameters.

Method files containing the instrument parameters have the name of the related analytical module. For example:

Table 5 Method file examples

HPCE1.MTH	Comprises the acquisition method for the Capillary Electrophoresis.
ADC1.MTH	Comprises the Agilent 35900 acquisition method. If two identical instruments are configured, the method files are called ADC1.MTH, ADC2.MTH.
DAMETHOD.REG	For data evaluation.
LALS1.REG	Comprises parameters for the Agilent 1100/1200 Series autosampler when a classic modular LC system is configured. The method files for the other Agilent 1100/1200 Series modules follow the same convention Lxxx1.reg where xxx is the module acronym.
AgilentSamplerDriver1.Rapid Control.xxx.xml	Comprises parameters for the Agilent 1100/1200 Series autosampler when a modular LC system is configured. Several .xml files are present for the various parts of the parameters (indicated by the xxx part of the file name). Similar .xml files are available for the other modules.

Editing Methods in Online Mode

When an online ChemStation is idle, you can edit all parts of a sequence method. When a sequence is currently running, you can edit all acquisition parameters and some of the Data Analysis parameters, such as the settings under Specify Report.

The changes are saved and immediately set into effect for the current run and all following sequence lines that include the same method. This means that you can also change the method during a sequence pause or a partial sequence.

Editing Methods in Offline Mode

You can edit a sequence method in an offline ChemStation, while the same method is used for a run in an online ChemStation. In this scenario, you can edit the Data Analysis part in the offline session. Once you save the changes in the offline session, the changed DA settings will be used for the next Data Analysis of the current sequence run within the online session.

Method updates concerning calibration are not taken into account. Also history entries are not merged, that is, if a method is running in an online session, and you change it in both online and offline session, the audit trail of the method will only contain the changes made in the offline ChemStation.

NOTE	If the same method is loaded in online and offline ChemStation, you can only edit the method offline during the sequence run. Method editing in the offline ChemStation is not possible if the online ChemStation is idle.

Administrating Methods

Methods Tree in ChemStation Explorer

The Methods Tree in the ChemStation Explorer is divided into two parts. The upper part shows the methods contained in the currently loaded result set. The lower part shows the methods in the master method directories, which you configure in the **Preferences** dialog.
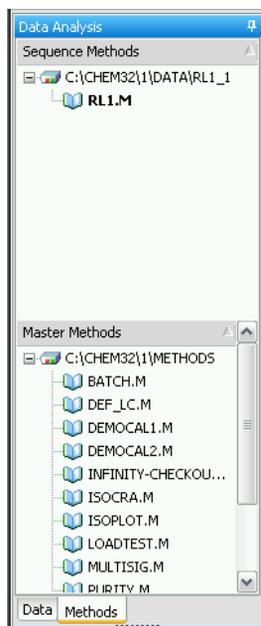

Figure 3   Methods Navigation Tree

The currently loaded method is always shown in bold.

By Drag & Drop you can easily copy master methods to sequence methods. The entire method (DA parameters and ACQ parameters) will be copied to the result set.

Viewing the Acquisition Method

You can access the Acquisition Method Viewer via the menu **Instrument >
Acquisition Method Viewer...** in the **Method and Run Control** view. The Acquisition
Method Viewer is available for both Online and Offline ChemStation sessions.

With the Acquisition Method Viewer, you can check the acquisition parameters
stored in a method, independently of the current instrument configuration.
The dialog shows the instrument configuration at the time when the method
has been saved in ChemStation. Data analysis parameters are not shown in
this viewer. The Acquisition Method Viewer does not allow the user to make
any changes to the loaded ChemStation method.

The Acquisition Method Viewer shows the method settings in read-only mode.
It does not provide functionality to edit and save methods.

NOTE	This dialog shows method settings for instruments or modules with RC.Net drivers only. It does not show method settings obtained from classic drivers.
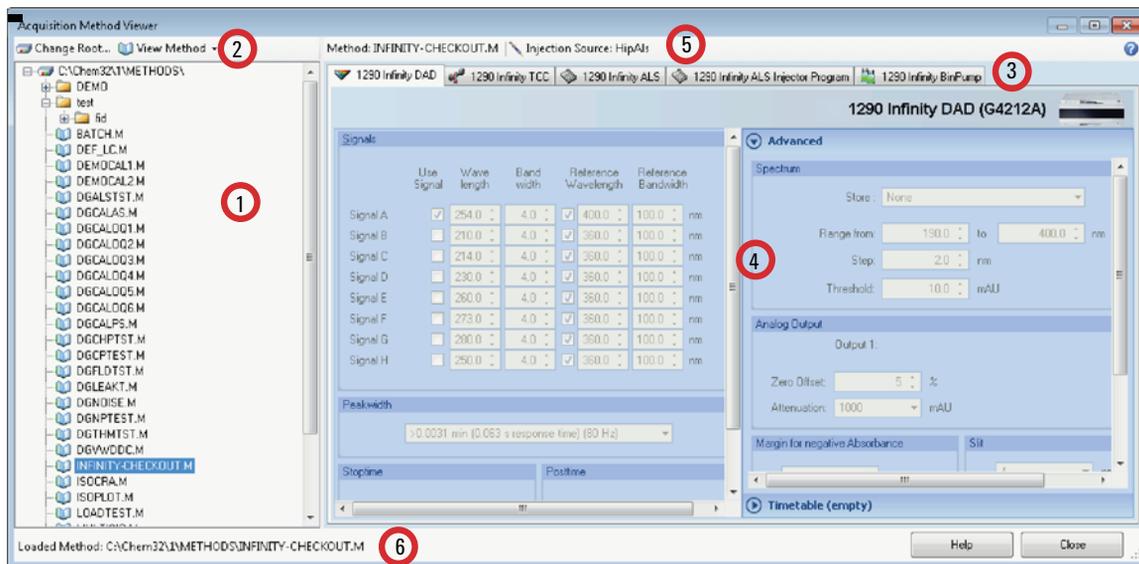
Figure 4 **Acquisition Method Viewer** dialog

1	Method browser
2	Toolbar
3	Module tabs (It shows method settings, pretreatment settings in a tab-control for all found modules in the used instrument configuration)
4	Method viewer area
5	Method Name, Injection Source Info
6	Status bar

In the method browser (1), the preferred method path is shown by default. Click **Change Root...** in the toolbar to select a different directory.

The toolbar (2) offers the following **View Method** options:

- **View with Original Configuration...**: Load the settings as stored in the method to the instrument.

- **View with Instrument Configuration...**: Apply the stored method to the current instrument configuration. This option is available for Online instruments only. The stored method might not be consistent with the current instrument settings; if possible, the settings are then automatically adapted, otherwise, you can click **Resolve Settings** and check the details in the **Method Resolution Info** dialog. This dialog shows the list of inconsistencies and the differences between unresolved and resolved method.
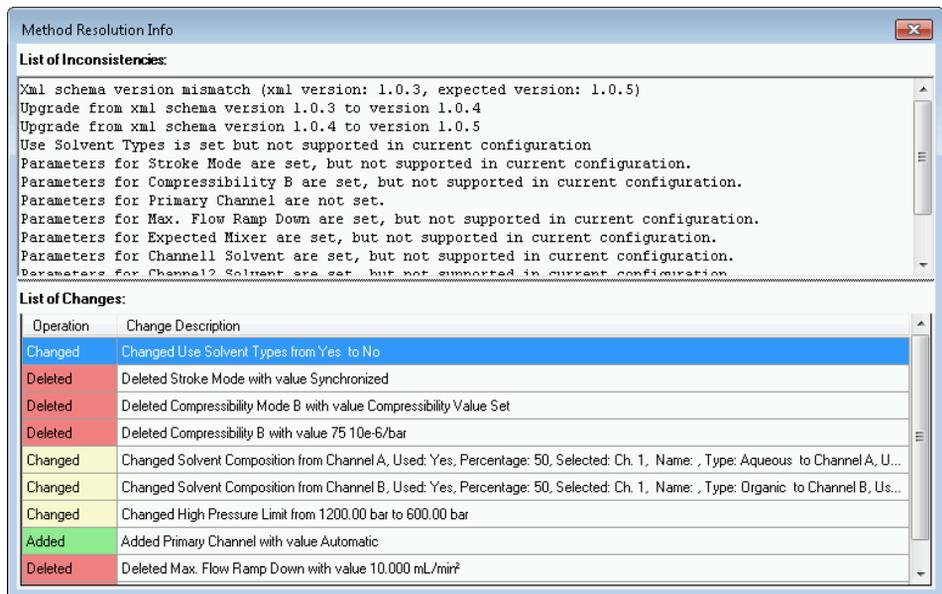

Figure 5   **Method Resolution Info** dialog

Updating DA Parameters in the Master Method

The **Update Master Method** option is available from the **Method** menu and from the context menu of the sequence method in the ChemStation Explorer. The exact behavior of this function depends on the currently selected mode. In all cases, this function updates the data analysis parameters of the target method.

> **NOTE**　It is important to note that this feature updates the data analysis parameters *only* of the target method, and that it overwrites *all* data analysis parameters.

Updating a Master Method in Reprocess or Recalculate Mode

In this mode, the **Update Master Method** command is only active for sequence methods of a result set. You can update the master method that you referred to when creating the sequence. The precondition is that the master method still exists in the master methods directory (the master method must have the same name as the sequence method).

You can also configure the sequence parameters to automatically execute this function during each sequence acquisition or reprocessing. For more information, refer to "Administrating Methods" on page 48.

Updating a Master Method in Last Result Mode

In this mode, the **Update Master Method** command is active for both sequences and single samples. You can transfer the current data analysis parameters to the master method last used for data analysis. This method is shown in the **Analysis Method** column of the Navigation Table.

The command is available under the following conditions:

- The method file exists in the given location (that is, the name and the entire path must match).
- For sequences: The sequence run has been manually analyzed with a master method (not with the sequence method).

Updating Any Master Method in Last Result Mode

In Last Result Mode, you can transfer the data analysis parameters to any master method, independently of the master method related to the current sequence or single run. To update any master method, select **Menu > Update any Master Method ...**, and select a method from the **Choose Master Method to update** dialog. The data analysis parameters will then be copied to the selected master method.

Updating Methods

With the **Update Methods** dialog (see figure below), you can copy methods from the master method directory to the result set and vice versa. In both cases, the entire method is copied (DA parameters and ACQ parameters).

You can open the dialog from the **Method > Update Methods...** menu or from the context menu of the sequence method in the ChemStation Explorer. The function is available for result sets in Recalculate and Reprocess mode.
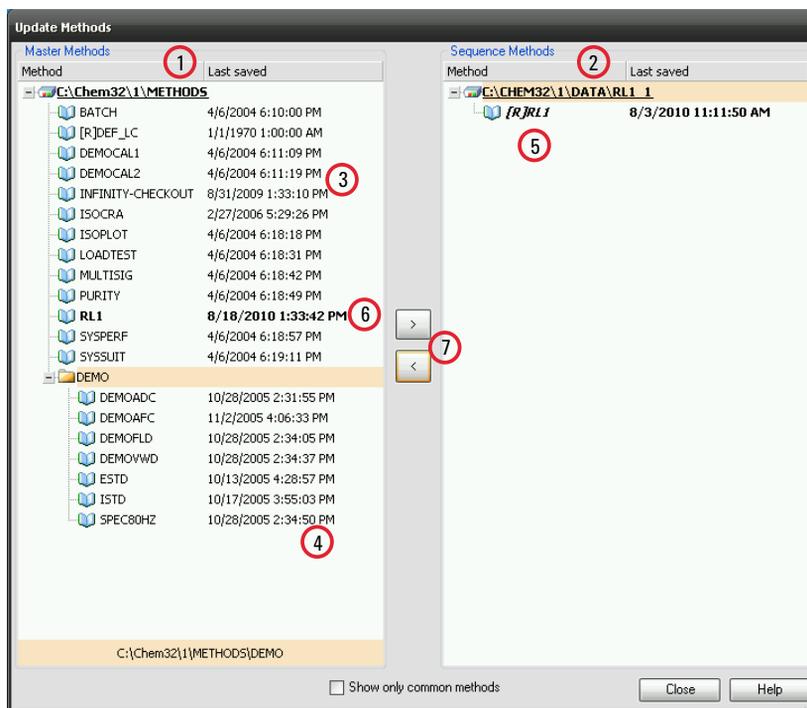
Figure 6   **Update Methods** dialog

1   On the left side, you see the methods in all master method directories (as configured in the Preferences).

2   On the right side, you see the methods in the currently loaded result set.

3   For each method, you see the date when it was last saved. The tooltip of the date shows the method's last history entry.

4   Methods can also be stored in subfolders of the master method directory.

5   Read-only methods have an [R] prefix. The currently loaded sequence method is shown in italics.

6   Methods that are common for the sequence result set and the master method pool are shown in a bold font. The methods are matched only by name; if a method name exists in more than one pool each of the instances will be considered common.

7   You can copy methods between a master method pool and the sequence result set either by using Drag&Drop or by using the < and > buttons. You cannot overwrite methods that are marked as read-only.

Saving as a New Master Method in Last Result Mode

You can save the data analysis parameters from DA.M as a new master method. However, the DA.M does not contain acquisition parameters. To provide a valid set of acquisition parameters for the new master method, you must therefore select another method as a template for the acquisition parameters (see figure below). The new master method will then contain your current data analysis parameters from the DA.M, and the acquisition parameters from the selected template method. The new method will be created in the folder where the acquisition template method is located.
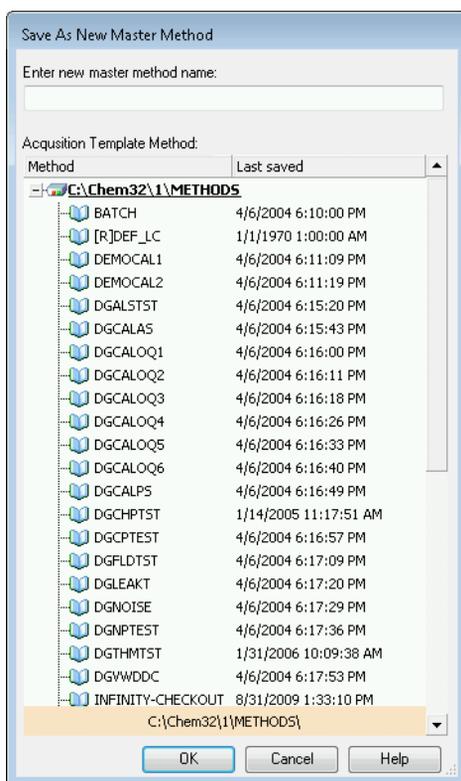

Figure 7   **Save as New Master Method** dialog

What Happens When a Method is Run?

The **Run Time Checklist** dialog box specifies the parts of the method to execute when a run is started.

There are eight parts to the run time checklist:

- pre-run command or macro,
- data acquisition,
- standard data analysis,
- analysis method for second signal (GC only),
- customized data analysis,
- save GLP data,
- post-run command or macro, and
- save copy of method with data (RUN.M).

When running a method the specified parts of the method defined in the Run Time Checklist dialog box are executed.

Method Operation Summary

The following list shows the flow of the method operation when all parts of the Run Time Checklist are selected.

1 *Prerun Command Macro*

Does a task before the analysis is started.

2 *Data Acquisition*

Does injector program.

Injects sample.

Acquires raw data.

Stores data.

3 *Save Copy of Method with Data* (RUN.M) - optional by Run Time Checklist

4 *Data Analysis (Process Data)*

Loads data file.

Integrates data file.

Identifies and quantifies peak.

Searches spectral library if available.

Checks peak purity if available.

Saves a copy of the method (DA.M) and prints report.

5 *Customized Data Analysis*

Executes your macros.

6 *Save GLP Data*

Saves binary register GLPSave.Reg

7 *Postrun Command Macro*

Does a task after completion of the analysis, for example, generates a customized report.

Figure 8   Method operation

The figure below shows an overview of the ChemStation status during the method operation, where all parts of the Run Time Checklist are selected.

NOTE	In case of the mode "Unique Folder Creation OFF" no DA.M is generated. For more details refer to "Preferences - Sequence Tab" on page 97.

Pre-run Command or Macro (Run Time Checklist)

If a pre-run command or macro is specified, it is executed before the analysis is started. This part is typically used for system customizing in conjunction with other software packages.

Data Acquisition (Run Time Checklist)

- All parameters are set to the initial conditions specified in the current method.
- If specified the injection program is executed and an injection is made from the currently defined vial.
- The monitor display shows the progress of the analysis including chromatographic/electropherographic information, and spectral data if available.
- Data are acquired and stored in a data file.
- When the Data Acquisition is finished, the acquisition parameters of the currently executed method are stored as ACQ.txt for the data file by default.

Data Analysis (Run Time Checklist)

When the stop-time has elapsed, the analysis is finished and all raw data is stored on the computer's hard disk. The data analysis part of the software starts when all the raw data is stored.

Integration

- Chromatogram/electropherogram objects in the signal are integrated as specified in the Integration Events dialog box.
- The start of the peak, the peak apex, retention/migration time and the end of the peak are determined.
- Baselines are defined under each peak to determine final peak height and area.
- The integration results are created as an Integration Results list.

Peak Identification and Quantification

- Using retention/migration times and optional peak qualifiers, the software identifies the peaks by cross-referencing them with known components defined in the calibration table.

- By using peak heights or peak areas the software calculates the amount of each detected component using the calibration parameters specified in the Calibration Table.

Spectra Library Search (for LC 3D, CE, CE/MS and LC/MS Systems Only, Available With Classic Reporting)

For all peaks that have UV-Visible spectra available, an automated search of a predefined spectral library may be done to identify the components in the sample based on the UV-Visible spectra. See *Understanding Your Spectral Module* for details.

Peak Purity Checking (for LC 3D, CE, CE/MS and LC/MS Systems Only)

For a peak with UV-Visible spectra, you can calculate a purity factor for that peak and store it in a register. Peak purity may be determined automatically at the end of each analysis as part of the method, if the Check Purity box is checked when specifying an automated library search or when selecting an appropriate report style. See *Understanding Your Spectral Module* for details.

Print Report

A report is generated with identities and amounts of components detected in the run.

Customized Data Analysis

Enables you to run your own customized macros to evaluate your analytical data.

Save GLP Data

Saves the binary register GLPSave.Reg together with the data analysis method in the default data file subdirectory. This feature is designed to help prove the originality of the data and the quality of the individual analysis.

The GLPSave.Reg binary file contains the following information in a non-editable, checksum-protected, register file:

- key instrument set points (can be graphically reviewed),
- chromatographic or electropherographic signals,
- integration results,
- quantification results,
- data analysis method, and
- logbook.

These data are saved only if the Save GLP Data feature is activated by checking the check box in the runtime checklist. You can review, but not edit, GLP data in the data analysis menu of ChemStation.

Postrun Command or Macro

If a postrun command or macro is specified it is executed after the data evaluation, for example, copying data to a disk for data backup.

Save Copy of Method with Data

This is done after data acquisition, and only if **Save method with Data** is activated in the Run Time Checklist. It copies the method used for acquisition to the data directory, named as RUN.M. RUN.M contains DA and ACQ parameters. It is read-only and thus provides a means for you to reconstruct the analysis in the future even if the method changes in the meantime. You can see how changes in the method or in selected parameters have influenced your analysis, which can help you to optimize it.

Save Copy of Method as DA.M with Data (ChemStation Default)

Independently of the marked items in the Run Time Checklist, a copy of the data analysis parameters of the executed method is saved as DA.M along with the report in the data file. This is done at the end of the *Standard Data Analysis* part, and also when you create a report in the Data Analysis view.
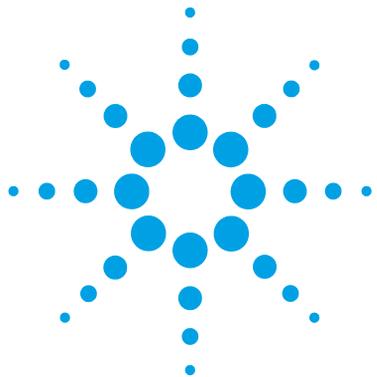
3
Data Acquisition

This chapter contains an introduction to the process of acquiring data.

Agilent Technologies

What is Data Acquisition?

During data acquisition, all signals acquired by the analytical instrument are converted from analog signals to digital signals in the detector. The digital signal is transmitted to ChemStation electronically and stored in the signal data file.

Path Selection

Starting with ChemStation B.02.01, flexible data storage for single runs and sequences allows you to specify various saving locations without reconfiguration. The **Paths** tab in the **Preferences** dialog box in the **View** menu gives you the opportunity to add multiple paths in addition to the default path C:\chem32\x\DATA (where x is the instrument number). Using the **Add** and **Remove** buttons, existing paths can be simply deleted, or you can navigate to a selected location and add the path to the new location into the **Preferences**. The default path cannot be removed from the list, but it can be changed in the **Configuration Editor**.
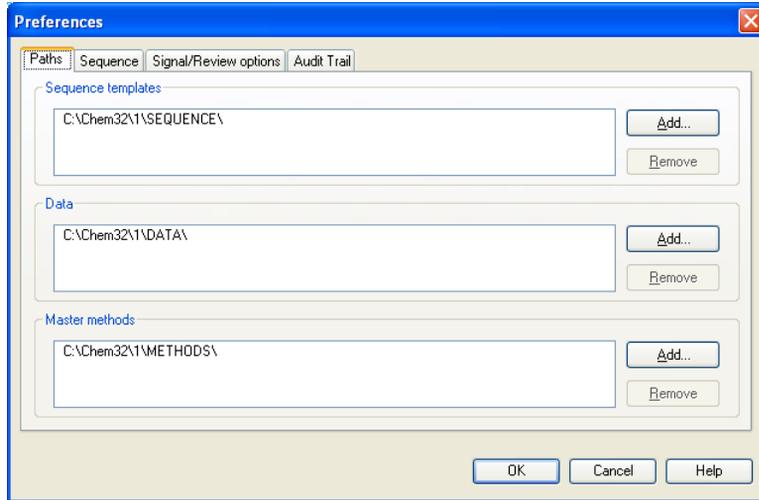
Figure 9 **Paths** tab in the **Preferences** dialog

All newly specified Data Paths are then available for selection in the **Sample Info** and **Sequence Parameters** dialog boxes when performing runs.
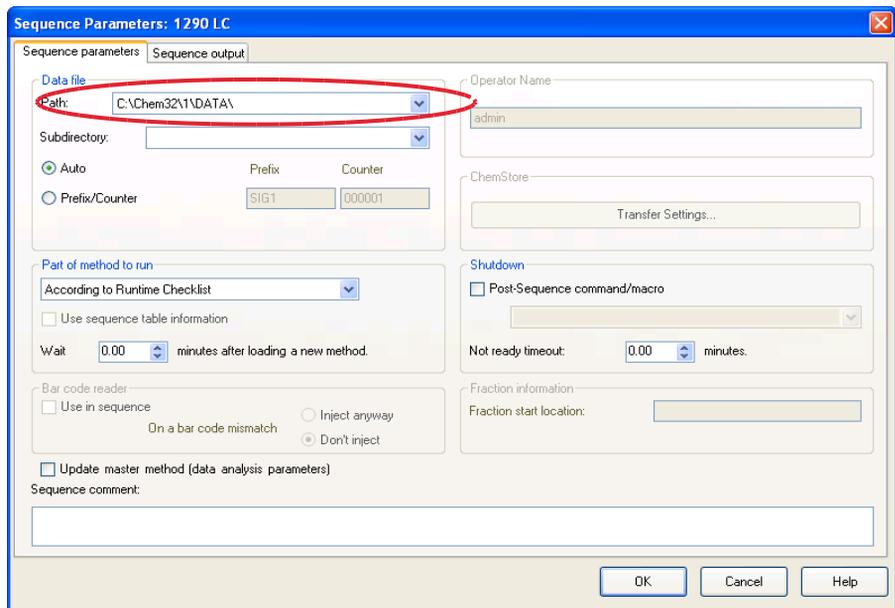

Figure 10 Data path selection in the **Sequence Parameters** dialog

Online Monitors

There are two types of online monitors, the online signal monitor and the online spectra monitor.

Online Signal Monitor

The online signal monitor allows you to monitor several signals and, if supported by the associated instrument, instrument performance plots in the same window. You can conveniently select the signals you want to view and adjust the time and absorbance axis. For detectors that support this function a balance button is available.

You can display the absolute signal response in the message line by moving the cross hair cursor in the display.

Online Spectra Monitor

The online spectra monitor shows absorbance as a function of the wavelength. You can adjust both the displayed wavelength range and the absorbance scale.

Logbook

The logbook displays messages that are generated by the analytical system. These messages can be error messages, system messages or event messages from a module. The logbook records these events irrespective of whether they are displayed or not. To get more information on an event in the logbook double-click on the appropriate line to display a descriptive help text.

Status Information

ChemStation Status

The ChemStation Status window shows a summary status of the ChemStation software.

When a single analysis is running:

- the first line of the ChemStation Status window displays run in progress,
- the second line in the status window displays the current method status, and
- the raw data file name is shown in the third line together with the actual run time in minutes (for a GC instrument, files for front and back injector are also displayed).

The Instrument Status windows provide status information about the instrument modules and detectors. They show the status of the individual components and the current conditions where appropriate, for example, pressure, gradient and flow data.

Status Bar

The graphical user interface of the ChemStation system comprises toolbars and a status bar in the Method and Run Control View of ChemStation. The status bar comprises a system status field and information on the currently loaded method and sequence. If they were modified after loading they are marked with a yellow cogwheel. For a Agilent 1100/1200 Series module for LC a yellow EMF symbol reminds the user that usage limits that have been set for consumables (for example, the lamp) have been exceeded.

System Diagram

If supported by the configured analytical instruments (for example, the Agilent 1200 Infinity Series modules for LC or the Agilent 6890 Series GC) you can display a graphical system diagram for your ChemStation system. This allows you to quickly check the system status at a glance. Select the System Diagram item from the View menu of the Method and Run Control View to activate the diagram. It is a graphical representation of your ChemStation system. Each component is represented by an icon. Using the color coding described below the current status is displayed.

Table 6   Colors used to indicate the status of the module or instrument

Color	Status
dark gray	offline
light gray	Standby (e.g. lamps off
yellow	not ready
green	ready
purple	pre-run, post-run
blue	run
red	error

In addition, you can display listings of actual parameter settings. Apart from a status overview, the diagram allows quick access to dialog boxes for setting parameters for each system component.

See the instrument part of the online help system for more information on the system diagram.

4

Automation/Sequences

Agilent Technologies
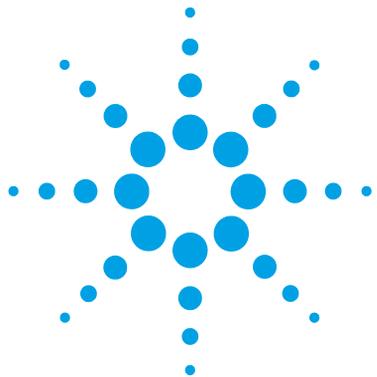
This chapter describes the concepts of automation. It explains how to work with sequences in ChemStation, what happens when a sequence is run and how to customize sequences.

What is Automation?

Automation is the unattended analysis of more than one injection.

The sequence part of the ChemStation software allows you to automate acquisition, data evaluation, and report generation.

What Are Sequences and Sequence Templates?

A sequence is a series of instructions that automates the analysis of samples. It can be used to automatically inject each sample, and to acquire and analyze the data according to the method specified for that sample. Each sample vial in a sequence may be analyzed with a different analytical method and, thus, use different sets of chromatographic/electropherographic conditions and evaluation parameters.

ChemStation introduces two data storage modes, to allow you to choose the data storage model that matches your workflow. These modes have influence of the sequence usage:

- **Unique Folder Creation** ON
- **Unique Folder Creation** OFF

Unique Folder Creation ON for consistency of sample data uses sequences as "sequence templates" that can be used to run the acquisition multiple times; however, these templates are not used for reprocessing in **Data Analysis**. When the sequence template is run, a result set is created, containing all related files. If the sequence template is reused, a new result set is created for each reuse

Unique Folder Creation OFF stores all data in one directory. The sequence *.s files are not used as sequence templates, so re-executing a sequence again might overwrite present data, if the data directory is not changed from the user.

The available sequence/sequence templates (*.s) are visible in the ChemStation Explorer. For quick and easy navigation, you can add additional sequence/sequence template locations to the ChemStation Explorer selection tree using the **Paths** tab of the **Preferences** dialog box.

Sequence Parameters

The **Sequence Parameters** dialog box contains information common to all sample vials in a sequence. Use this dialog box to:

- select the data directory using the **Path** combo box, and
- specify how the sequence processing should be done by choosing particular part of Methods and Run parameters.

For example, you can choose to either:

- execute the run time checklist,
- do acquisition only, or
- do reprocessing only (for data acquired till ChemStation Rev. B.01.03. or for data acquired with option **Unique Folder Creation OFF**).

Sequence data acquired with ChemStation revisions up to B.01.03 or acquired using the options **Unique folder Creation OFF** need to be reprocessed using the **reprocess** option in the **Method and Run Control** view.

Sequence data acquired with ChemStation revisions B.02.01 and higher need to be reprocessed using the **reprocess** option in the **Data Analysis Navigation table**.

If the **reprocess** option is selected, you have the choice to use the sample data defined when the sample was originally analyzed, or by activating the **Use Sequence Table information** check box, you can use updated sample data by entering new data in the sequence table:

- specify what happens when the sequence is finished using the **shutdown** parameters, and
- specify if the barcodes should be used in the sequence and how to handle a barcode mismatch, assuming that a barcode reader is installed on the system.

Sequence Table

The sequence table determines which methods are used to analyze the sample vials and the order in which the vials are analyzed. This table also contains information about each sample, including the name, quantification parameters, and recalibration parameters.

The injector group box is displayed for instruments that support dual sampling (GC). Selecting **Front** or **Back** displays the lines in the sequence table and the currently running status for that injector.

For a description of the columns in this table and how they interact with information stored with the method, see the online help reference.

Creating Sequences (Sequences and Sequence Templates)

Use the sequence table to specify the samples, methods, and vials to run in the sequence. The sequence table lists each sample in the sequence in the order it will be run and contains the necessary vial, method, and calibration information for each sample.

Using the Sequence Table Editor

If you would like to change the view and content of your sequence table, you can open the Sequence Table Editor by clicking **Configure Table** in the lower right corner of the Sequence Table. The Sequence Table Editor opens and allows you to specify if a certain column is shown within the Sequence table. In addition the column widths can be defined for each sequence table column. Depending on the installed software packages, additional column fields will be added, for example, the **Target Mass** field if an LC/MS is installed.

Using the Insert Vial Range Button

If you have many samples that use the same method, you can quickly enter these samples into the Sequence Table using the Insert Vial Range feature. This feature copies the method name, vial range, number of injections per vial, and if you specify the Sample Amount, ISTD Amount, Multiplier, and Dilution. The system then enters the information for each vial in the range into the sequence table.

Using the Append Line Button

To append a new blank line to the end of the sequence table, select the Append Line button.

Using the Custom Fields Button

If custom fields have been set up in the method(s) used in the sequence table, select the Custom Fields button in order to edit the custom fields values for each sample (sample related custom fields) or for each compound in the method of a sample (compound related custom fields).

Easy Sequence

Overview

Easy Sequence is a user interface for quick and easy setup of sequences from templates. The template specifies parameters that should be viewed or edited by the user. Calibration setup provides an easy to use drag and drop interface to specify calibration types and sample positions, and displays an overview of the sequence. With **Easy Sequence**, multiple sequences can be submitted to the Run Queue to be run on the data system.
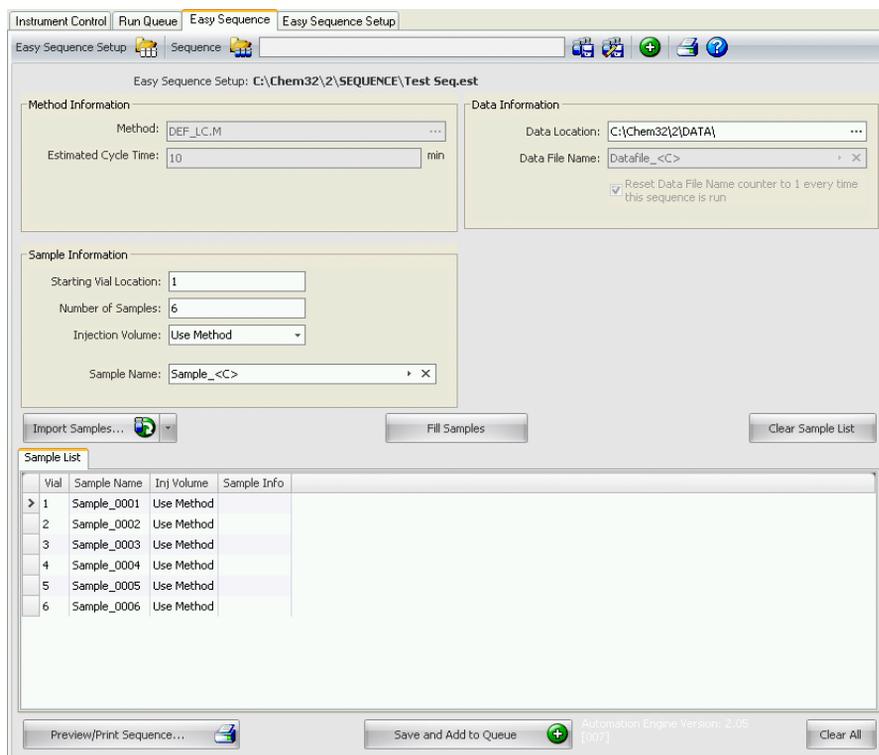
Figure 11   Easy Sequence tab

Using Easy Sequence Tab (Sequence)

Easy Sequence tab is used to create a sequence from the template created in the **Easy Sequence** Setup. Samples saved as a CSV format can also be imported.

To define a Sequence

1 From the **Easy Sequence** tab, open a template by clicking on the **Open Easy Sequence Setup** icon.

2 Make updates if required. This may include sample vial locations, calibrant vial locations, data, or sequence location. Parameters available for editing depend on the template configuration.

3 If the pre-filled samples do not match new sample locations, click **Fill Samples** to refill the table.

4 Click **Preview/Print Sequence...** to preview the Sequence

5 Save the sequence.

| HINT | The sequence can be edited as long as its status is **Pending** in the queue. |

6 Click **Save and Add to Queue** to submit the Sequence to the Sequence Queue.

To import Sample Data

Sample data set can be imported into **Easy Sequence**. Before importing samples, the CSV file must be set up and formatted properly. Refer to online help for more information on how to create a CSV sample data file.

1 From the **Easy Sequence** tab, open a template by clicking on the **Open Easy Sequence Setup** button

2 Click **Import Samples...**

3 Select the CSV file you want to import.

All valid fields are imported.

| NOTE | To import sample data to the **Back Sample List**, make sure that the **Back Sample List** is selected and displaying before pressing the **Import Samples** button. |

4 Verify the fields by reviewing the Sample List.

Using Easy Sequence Setup Tab (Template)

Easy Sequence Setup is used to create templates which are the starting point for creating sequences. There are two panels: Samples and Calibration. The **Samples** panel specifies the method, sample, data, and sequence information. The template is also used to specify what parameters are hidden or read-only. The **Calibration** panel provides a graphical interface to configure and view calibration runs. It provides an easy-to-use drag and drop interface to specify calibration types, cyclic and bracketed, and sample positions.

Creating an Easy Sequence template:

1 From the **Easy Sequence Setup** tab, select the **Samples** panel. Open an existing template or create a new template.

2 Select the **Method**. Dual injection options will be shown if the method's injection source is Dual. A back analysis method can be specified for the back signal. The method is the only parameter required for a template.

3 If desired, enter the estimated duration (in minutes) of a sample run. This is the time measured from when a sample starts to when the next sample starts. This parameter is used to estimate the total expected duration of your sequence. Leave this field blank if you do not want to use the Estimated Cycle Time feature.

4 Specify the **Starting Vial Location**, **Number of Samples**, and the **Sample Name**.

5 Select the **Data Location**

6 Select the **Sequence Location** and specify the **Sequence Name**.

7 Enter any comments for the template.

8 Specify which parameters are hidden or read-only. Enter a default value for **injections/vial**, **sample amount**, **ISTD amount**, **injection volume**, etc. This helps to minimize the chance for errors when creating a sequence in the **Easy Sequence** Tab.

9 Save the template.

To define Calibrations:

The method used in the template should have been calibrated to the necessary levels.

1 From the **Easy Sequence Setup** tab, select the **Calibration** panel

2 Select **Cyclic**, **Bracketing**, or **Simple Calibration** from the **Calibration Mode** drop down list.

3 The **Sequence Diagram** has the following sections:

   • **Sequence Start**

   • **Bracketing/Cyclic**

   • **Samples/Injections**

   • **Sequence End**

4 In the **Samples** area for the Sequence, set the **Calibration Interval** based on number of samples or number of injections.

5 Setup the **Sample type**, **Blank**, **Calibrant**, or **QC Sample** by dragging the icon from the **Sample Type** area to the **Sequence Diagram** section.

6 Setup the parameters for each Sample type and set to **Hide** or **Read-Only**.

7 Verify the calibration mode in the **Easy Sequence** Overview.

8 Save the template.

Working with Sequences (Sequences and Sequence Templates)

Sequences (Sequences and Sequence Templates) are accessed and created from the Sequence menu. Sequences may be created and saved in the same way as methods. When you save a sequence, a file is created with a .S extension. When you want to edit or use the sequence again, you access it by, for example, using the Load Sequence item in the Sequence menu.

Data Acquisition in a Sequence

In order to run a sequence, appropriate pre-defined methods must be available. These are the master methods as outlined above. Typically, master methods and sequence templates are worked on in the **Method and Run Control** view of ChemStation. For this reason, in **Method and Run Control** view, the ChemStation Explorer provides access to master methods and sequence templates.

The sequence template references these methods in the sequence table.

As explained previously, when a sequence is run with sequence template <sequence_name>.S, and the master method <method_name>.M is used, a new folder is created that contains all resulting files from the sequence run ("result set").

The location of this folder is determined by the settings in the **Sequence Parameters** dialog box; the naming of this folder is determined by the **Sequence** tab of the **Preferences** dialog box. By default, the name is <SeqName> <Date> <Time>, but it can be configured by using tokens, or you can manually enter any name. For more information on using tokens, see "File Names and Tokens" on page 16. You can use the following tokens:

- **Current date**
- **Current time**
- **User name**
- **Instrument name**
- **Sequence name**

- **Counter**

- **Computer name**

If the **Name Pattern** would not result in unique names for the result sets, ChemStation will append a counter to ensure uniqueness.
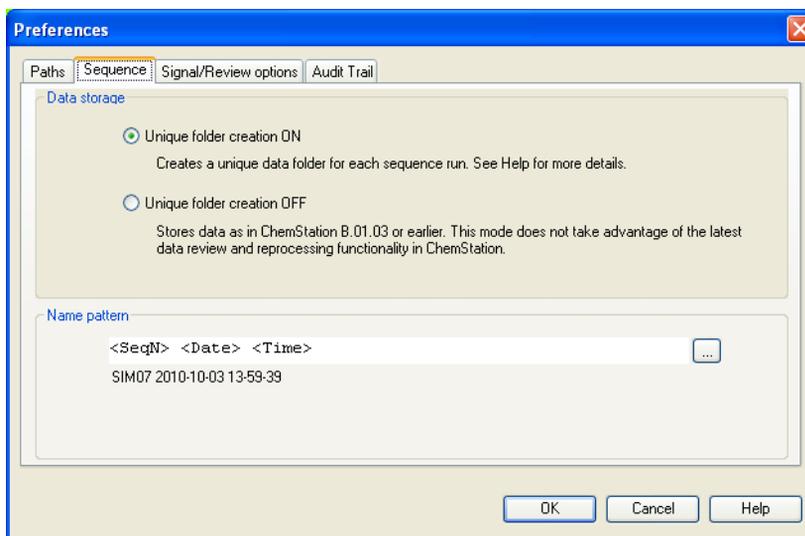
Figure 12   **Preferences** dialog / **Sequence** tab

At the start of an acquisition sequence, the method specified in the sequence table is copied from the master methods folder into the result set. In addition, a copy of the sequence is created and placed with the sequence log and the batch (*.b) file in the result set. All updates of the method (for example, updates of the calibration table) are written to this sequence method in the result set. If you use Intelligent Reporting, the report templates you selected in the Sequence Parameters or in the Method Properties are also copied to the result set. All necessary files are now available for future data review and reprocessing, without changes that were applied to the master method or sequence template for other sequence runs.

During acquisition, the data files are stored to the result set. Within each data file (*.D), a copy of the sequence method is saved for this specific run. The file ACQ.txt contains the acquisition parameters of the sequence method, preserving the state of the method as it was at the time of acquisition of the specific data file. The folder DA.M contains a copy of the data analysis parameters used in the sequence method.

With these files saved in the sequence folder, all data review and reprocess activities can be performed without altering the master method or the sequence template. If needed, method changes can also be saved to the master method again.

<table>
<tr><td>

NOTE

</td><td>

The result set must always contain the complete set of all data files (*.D). If you delete part of the data files, uploading the result set to central data storage will cause problems. If you need to shorten a sequence, create a self-assembled result set from the reduced set of sequence lines (see "Creating a Self-Assembled Result Set" on page 92).

</td></tr>
</table>

Data Acquisition of Single Runs

For single runs, the data file is saved directly into the respective subdirectory. Since only one method is employed for a single run, this method does not need to be copied into the subdirectory; all actions are performed directly with the master method. After the acquisition part of the method is completed, a copy of the acquisition parameters is saved into the file ACQ.txt. A copy of the data analysis parameters is saved into the data file directory (DA.M) after the data analysis part of the master method has been executed.

Automatic Update of Master Methods

With this function, ChemStation automatically updates the data analysis parameters of the master methods that you copied into your result set. You can use this function for example to update the calibration tables of master methods after reprocessing a sequence with recalibrations.

You can activate this function in the **Sequence Parameters** dialog (see figure below). During acquisition, ChemStation updates the data analysis parameters of the master methods for all sequence methods in the result set.

The data analysis parameters of the master methods are also updated after reprocessing the sequence. The precondition is that the corresponding master method (a method with the same name as the sequence method) still exists in the same master methods directory as at the time it has been copied to the result set.
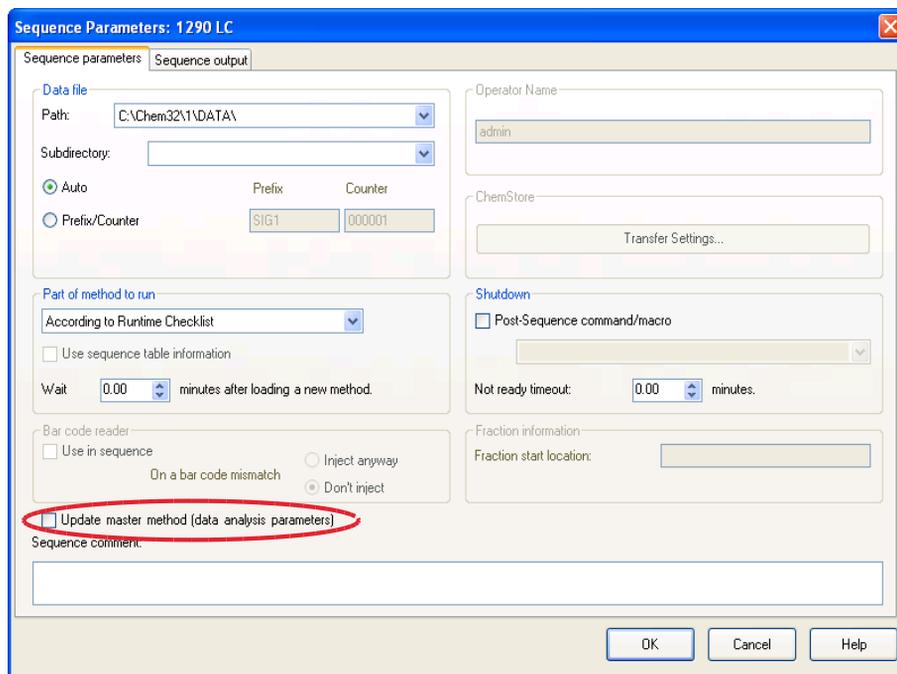
Figure 13   **Update master methods** option in **Sequence Parameters** dialog

NOTE	As this function will cost performance, it is not advised to use it if you have sequences with hundreds of methods.

Priority Samples

A currently running sequence can be paused after the current method is completed. The sequence can be paused to allow the analysis of a priority sample by the same or another method. The sequence can then be resumed, where the sequence continues with the sample at which the sequence was paused.

Sequencing with Control Samples

Control samples can be specified in the Sample Type field of the sequence table. The method used to analyze the control sample must contain a calibration table where control sample limits for one of the compounds are specified. If the control sample limits you specified are exceeded, the sequence is stopped and a message is written into the logbook. If you are using one of the ChemStation report styles, the control sample limits are also printed on the reports generated for these analyses. For more information on how to define a sequence with control samples, see the How To section of the online help.

Sequencing with Blank Reference Samples

Reference signals are required to evaluate the signal-to-noise ratio as defined by European Pharmacopeia. You can specify the reference data file in the sequence table by choosing the sample type **Blank** for the corresponding samples.

If you use multiple reference files, the order of the files is essential. ChemStation uses one reference file for all successive runs, until there is new reference file given in the sequence table. The reference file of a blank sample serves as its own reference. The following overview shows an example of a sequence containing two blank samples:

Table 7   Example for a sequence with blank samples

	Sample	Data file	Reference file
1	Sample1	DF01.D	
2	*Blank1*	*DF02.D*	*DF02.D*
3	Sample2	DF03.D	*DF02.D*
4	Sample3	DF04.D	*DF02.D*
5	*Blank2*	*DF05.D*	*DF05.D*
6	Sample4	DF06.D	*DF05.D*
7	Sample5	DF07.D	*DF05.D*

Please refer to the Reference Guide for details on the calculation of the signal-to-noise ratio.

Pausing a Sequence

The currently active run will be completed before the sequence pause.

During a sequence pause, the sequence table file name and the data file name cannot be changed. In the sequence table you can only change sequence lines that have not already been executed or change the vial number in the current sequence line. You can add, delete, and change sequence lines for future analyses.

For example, it may be necessary to edit an active sequence to add a new batch of samples. You can edit the sequence so these vials will be the next sample that ChemStation will process after the samples in the currently running sequence line.

Stopping a Sequence

The currently active run will be terminated immediately. However, the data analysis will still be executed for this run. A stopped sequence can never be resumed.

If you want to finish the current run before stopping the sequence, pause the sequence, wait for the run to be finished, and then stop the sequence.

Aborting a Sequence

The abort function terminates a currently active sequence immediately. No Data Analysis is executed.

Running a Partial Sequence

Result Set Selection for Partial Acquisition

If you use Unique Folder Creation ON (see "Preferences - Sequence Tab" on page 97, you can choose between the following options for a partial sequence acquisition:

- acquire the partial sequence into a new result set

or

- acquire the partial sequence into an already existing result set.

Acquiring the data files from a partial sequence execution into an already existing result set may be useful in the following scenarios:

- A single data file (or several data files) has to be overwritten, for example, because an incorrect vial was used in the first place.
- Only the first part of the sequence has been executed in the first place, and the missing samples have to be added by executing partial sequence. This may occur in the case of an instrument failure during sequence acquisition.
- Additional lines have been added to the sequence template after acquisition of the already existing lines. The additional runs are to be added to the already existing data.

Therefore, when you select **Partial Sequence** from the **Sequence** menu, a dialog will come up providing you the option to either select an existing result set from a list or to create a new result set.
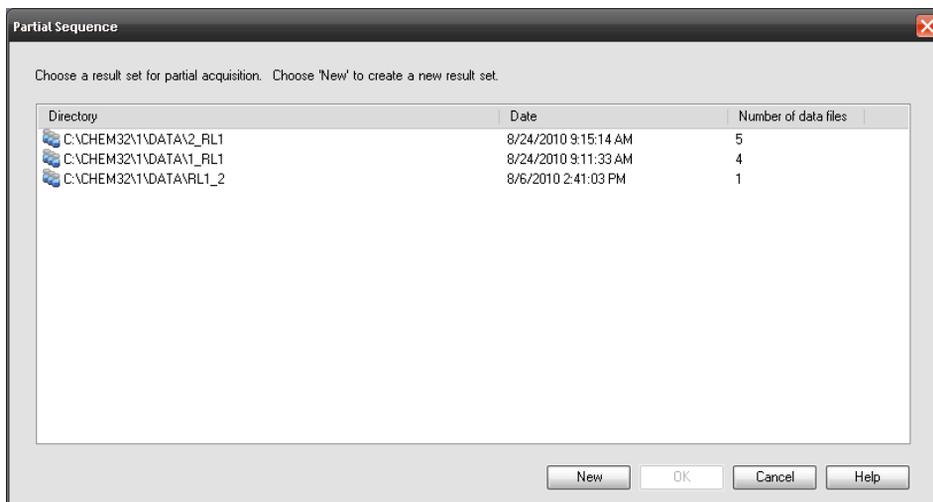
Figure 14   Partial Sequence dialog

However, in order to maintain consistency of the result set (so it can be completely reprocessed in **Data Analysis**), only those result sets are offered for partial acquisition that fulfill certain conditions:

- The name of the sequence template (source sequence) and the name of the sequence *.S file in the result set (target sequence) are identical.
- Both the data path and the subdirectory have to be identical for the sequence files.
- The number of sequence lines in the source sequence must be equal or higher than the number of sequence lines in the target sequence.
- For each line in the target sequence the sample type and the number of injections must be identical to the values in the corresponding lines of the source sequence.
- The data file naming scheme must be identical for the two sequence files.

After leaving this dialog with **Ok** (for selecting one of the existing result sets) or **New** (for creating a new result set), you can select the sequence lines to be executed during partial sequence.

Selection of Sequence Lines for Partial Sequence Acquisition

The system displays the **Partial Sequence** dialog box and allows you to select individual samples from the table for analysis. This dialog opens independently of the Unique Folder Creation setting.

A single run is shown on each line of the **Partial Sequence** dialog box. For each run, the vial, method, data file, and sample name are given. Additionally, encoded information about the sequence table and any calibration samples is shown in the Seq Tbl and Calib:RF:RT columns. See the online help for an explanation of these codes.

You can obtain a paper copy of the partial sequence by selecting the **Print** button.

With **Manual update ...**, you open the **Update Methods** dialog box, which allows you to manually synchronize the master methods and the methods used in the sequence template. With the **Automatic update for selected runs** option, you can update all sequence methods that are used in the selected runs with their corresponding master methods.

NOTE	Both acquisition and data analysis parameters are updated.

For example, the **Partial Sequence** dialog may look like the following. You can mark specific samples for processing.

Figure 15   Partial Sequence dialog box

Creating a Self-Assembled Result Set

Using the **Sequence > Create New Result Set** command in the **Data Analysis** view, you can create a new self-assembled result set from the data currently shown in the Navigation Table. Self-assembled result sets are helpful, for example, in the following scenarios:

- You want to combine single samples, sequences, or a combination of both in order to reprocess them with a specific method.
- You want to shorten a sequence.

To assemble a new result set

1 Add the required data files into the Navigation Table.

2 In the Navigation Table, select all the data files that you want to include in your new result set.

3 Select **Sequence > Create New Result Set** to open the **Create New Result Set** dialog box.

4 Select a method to be associated with the new result set.

5 Specify a folder for the new result set.

6 Sort the samples.

The names of output data files are automatically updated. If required, you can restore the initial order of the samples using the ⬒ (**Restore initial order**) button.

Note that the position of a blank file is relevant for the evaluation of the signal-to-noise ratio as defined by European Pharmacopeia. See also "Sequencing with Blank Reference Samples" on page 87.

7 Confirm the settings to assemble the list of data files into a result set in the specified folder.

Sequence Log File

A sequence log file is produced that indicates what has happened during the running of the sequence. It is useful for identifying when errors occurred if the sequence is running unattended or overnight. The name of the logbook file always has the .log extension. The logbook file is located in the directory where the data of the sequence is stored.

What Happens When a Sequence is Run?

Starting a Sequence Using Unique Folder Creation ON

The system creates a result set based on the path definition in the sequence parameters and the sequence preference settings. The sequence template *.s, all methods defined in the sequence table belonging to this particular sequence are copied to the result set. If you use Intelligent Reporting, all report templates *.rdl that are defined in the method or in the sequence template are also copied to the result set. The system continues to work with these files during acquisition. Starting the sequence, the method of the corresponding sequence line is loaded within ChemStation from this result set.

Starting a Sequence Using Unique Folder Creation OFF

Starting a sequence, the system is loading the sequence *.s file and based, on the entry in the sequence table the corresponding method of the sequence line is loaded within ChemStation. In contrast to the second data storage mode **Unique Folder Creation** ON, no result set is created. Sequence and methods will remain in their master directory.

Further Executed Steps During Sequence Execution:

The following steps will be repeated for each executed sequence line:

- If equipped with an autosampler, the ChemStation software first locates the sample in the autosampler according to the number entered in the vial column
- The instrument is loaded with the method parameters.
- The pre-run macro is executed.
- The sample is then injected into the instrument (manually or automatically).
- The data is acquired.
- The method data evaluation is done. Integration, quantification, and reporting, including any user specified macro. If mode **Unique Folder Creation** ON is used, the system stores an additional methods DA.M during the run.

- The post-run macro is executed.
- During the entire process, ChemStation tracks the sequence's progress in real time and produces a sequence log file.
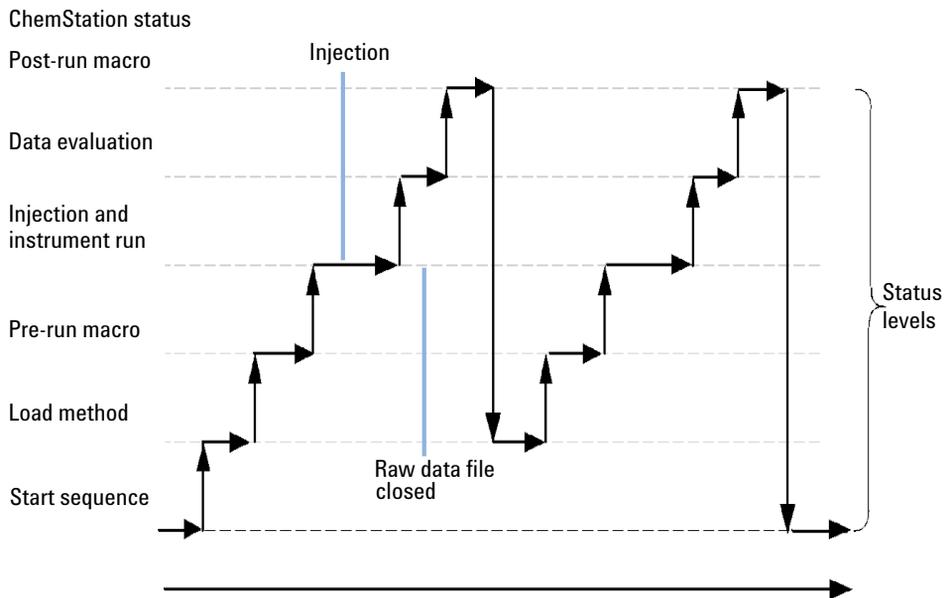
Figure 16   Sequence Status

Sequence Data File Structure

Preferences - Sequence Tab

On the **Sequence** tab in online sessions, the user has the choice of two different data storage models. These modes define how the sequence data are stored in ChemStation.
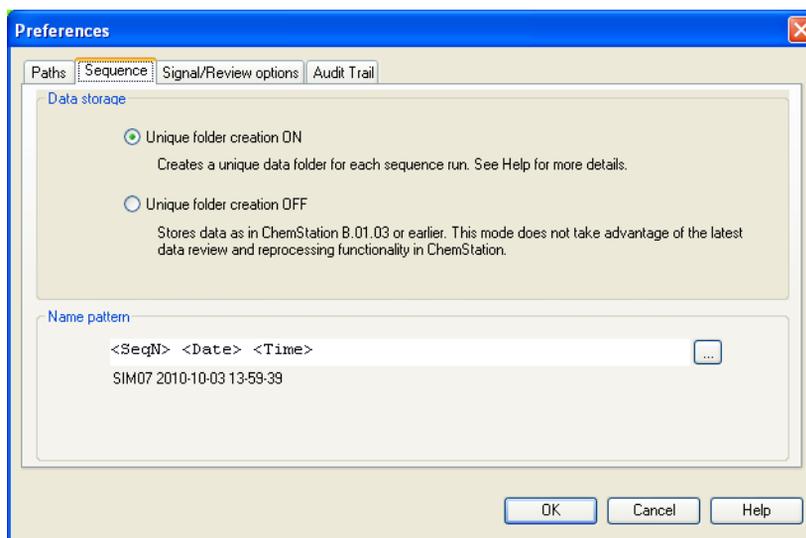
Figure 17   **Preferences** Dialog / **Sequence** Tab

> **NOTE**   Switching Unique Folder Creation on or off only affects future acquisitions, but does not change the data organization of already acquired data.

> **NOTE**   We strongly recommend to decide between the two modes at the beginning of your work and not to switch between them.
>
> Switching Unique Folder Creation off is not supported if your ChemStation is connected to a central data storage system.

Unique Folder Creation ON

In this mode of data storage, there is a robust and permanent link between the raw data and the method. Each data file, whether acquired within a sequence or as a single run, has a link to the method that was used for data analysis.

The sequence data are stored in a result set using a unique result set name. You can specify the naming conventions (Name Pattern) for these result sets in the **Sequence** tab of the **Preferences** dialog box. If no name pattern is specified, a default sequence name pattern is used. The **Sequence** Tab is used only for data acquisition and therefore is present only for online systems.

The sequence name pattern can contain various sections. The system creates a name for your result set that is determined by the sequence name pattern sections you select. All data files, methods, the sequence logbook, the <sequence_name>.s file and the <sequence_name>.b file belonging to this particular sequence are stored in the result set. The result set is created when the sequence is started.

The sequence (*.s) files are used as sequence templates, and this concept allows you to run any sequence file multiple times without overwriting existing data or changing the sequence parameters. If neither counter nor time is used in the sequence name pattern, the system introduces a counter automatically to avoid overwriting data. For the second, third and all subsequent sequences using the same sequence template, a counter is added to the result set name.

Unique Folder Creation OFF

In this mode of data storage, the method name is the only link that exists between the data file and the method that was used to acquire and process it. No copies of the method are saved with the sequence or with the data file; if the method is changed, or a new method with that name is created, then the sequence cannot be exactly reproduced. The sequence data files are stored in accordance with the parameters specified in the Data File group of the **Sequence Parameters** dialog box; the sequence naming facility in the **Sequence** tab of the **Preferences** dialog box is disabled in this mode. This mode of data storage is identical to ChemStation revisions prior to B.02.01 and therefore cannot take full advantage of the latest Data reviewing/reprocessing functionalities in the **Data Analysis** view of ChemStation.

NOTE   Sequence data acquired using the option **Unique folder Creation** OFF need to be reprocessed using the reprocess option in the **Method and Run Control** view.

NOTE   If you use ChemStation with central data storage, the preference mode **Unique Folder Creation** must be ON. With central data storage, the option **Unique Folder Creation** OFF is disabled.

Selecting **Unique Folder Creation Off** has the following impact on data storage:

- Sequence data is not acquired into a result set, but directly into the subdirectory as specified in the Sequence Parameters (see "Sequence Parameters" on page 75). Therefore, the sequence name pattern is grayed out on the **Sequence** tab of the Preferences dialog.

- This means that for two or more sequence acquisitions the data may be acquired into the same subdirectory.

- No sequence methods (.M) or copies of the sequence file (.S) are stored with the data, but only the sequence logfile and the batch file (.B). This means only the methods and sequences in the paths specified in the **Preferences** dialog (see "Path Selection" on page 64) are available. They have to be used for acquisition as well as for data review and reprocessing. Sequence or data file specific method changes can only be stored by saving the method with different name. Otherwise these changes are also applied to the acquisition method.

- When a sequence acquired with Unique Folder Creation Off is loaded into the Navigation Table, the reprocessing mode in the **Data Analysis** view is not available (Figure 18 on page 100). Sequences that have been acquired with Unique Folder Creation Off can only be reprocessed in **Method and Run Control** view using the **Reprocessing only** option in the **Sequence Parameters** (Figure 19 on page 100).

Figure 18   Navigation Table for sequences acquired with **Unique Folder Creation** switched off
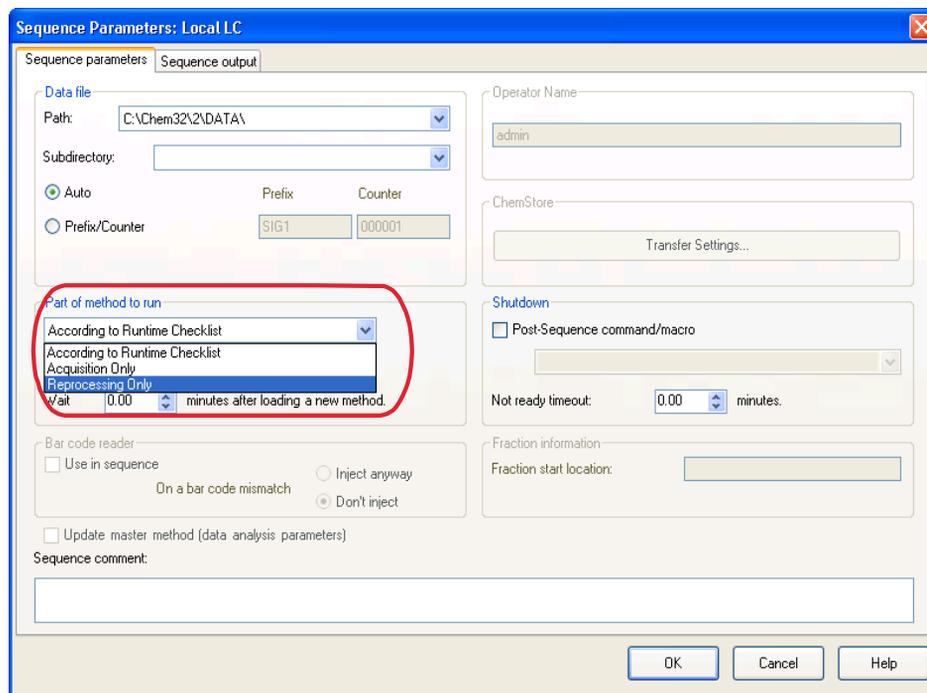
Figure 19   Reprocessing of sequence data acquired with **Unique Folder Creation** off

Data File Structure With Unique Folder Creation ON

There is a strong link between the raw data and the method, as shown in the figure below.
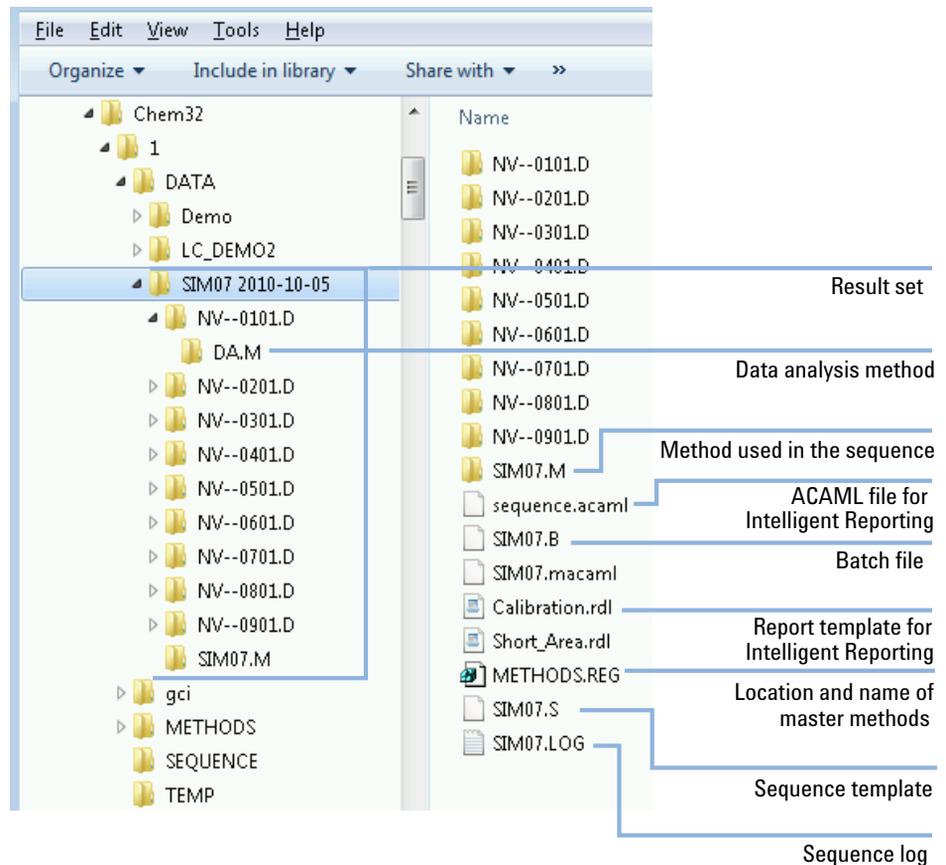
Figure 20   Sequence Data File Structure "Unique Folder Creation ON"

> **NOTE**   The result set must always contain the complete set of all data files (*.D). If you delete part of the data files, uploading the result set to central data storage will cause problems. If you need to shorten a sequence, create a self-assembled result set from the reduced set of sequence lines (see "Creating a Self-Assembled Result Set" on page 92).

Data File Naming in a Sequence

Data file naming in a sequence can be done in the following ways:

- automatic,
- manual, or
- prefix/counter.

Automatic Data File Naming in a Sequence

Sample Vials

For example 017-0103.D

where:

- The first three digits are the vial number, for example, 017.
- The fourth digit in liquid chromatography and capillary electrophoresis is a separation hyphen (-); in a gas chromatograph, this will be either (F) for front or (B) for back.
- The fifth and sixth digits are the sequence line that defines the method used, for example, 01 for the first sequence line.
- The seventh and eighth digits are the injection number for this vial by the method, for example, 03 for the third injection.

Blank Runs

For example NV--0499.D

where:

- NV stands for no vial.
- - is a separation hyphen.
- 0499 is the 99th blank run of sequence line 4.

Entering Data File Names Manually

One of the columns in the sequence table is named **Datafile**. When it contains no entry, the data file naming scheme specified in the Sequence Parameters (automatic or prefix-counter) is used to create the data file name. If any text is entered into the **Datafile** column, ChemStation uses that text as the data file name for the run.

If more than one injection per vial is specified on a line with a manual data file name, ChemStation automatically truncates characters from the end of the name entered by the user and appends the injection number. This prevents the same data file name from being reused for multiple injections.

Using a Prefix/Counter to Name Data Files

If you use the Prefix/Counter to name data files, ChemStation generates a name for each analysis. For an instrument that supports dual signal analysis such as the GC, ChemStation will generate a name for each signal.

The sequence set up allows long filenames for the prefix/counter. The data file name defined by prefix/counter can have up to fifteen characters plus .d extension, so in total seventeen characters.

Following rules apply to the prefix/counter field:

* the counter itself can have a maximum up to 6 characters
* if a prefix provides less than nine characters for prefix, the counter is automatically extended to 6 digits
* the number given in the counter is the start number being incremented

Table 8   File names

Prefix	Counter	Results in file name
long	000001	long000001
longname	000001	longname000001
testwithalongna	1	testwithalongna1

Result Set Migration

ChemStation provides a tool to migrate non-result set data to result set format. To successfully perform this task, it is required that the original sequence file is still available. It must contain all the necessary sequence lines and follow the original data file naming scheme to reprocess all the data files of the sequence. In addition, all the methods in the Method column of the sequence table have to be available.

To perform migration,

start the **Result Set Migration** from the **Sequence** menu in **Data Analysis** view.
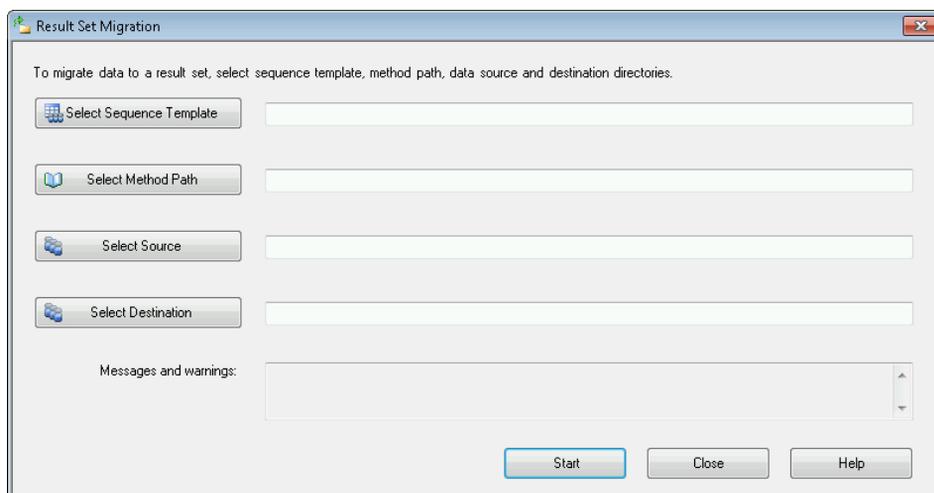
Figure 21   Result Set Migration

Fill in the following required fields:

Select Sequence Template: Select the sequence file (*.S) that contains the sequence table that matches the data set to be migrated.

Select Method Path: Select the directory where the methods are located that are referenced in the sequence table.

Select Source: Select the directory that contains the data files to be migrated.

Select Destination: Specify the path and name of the result set to be created. You may select an existing folder or create a new one.

When all fields are filled in, the migration can be started.

The following steps will be performed:

- The result set directory will be created.

- The sequence template will be copied to the result set. It will also be converted to a state where it is able to reprocess data files in **Data Analysis** view.

- The methods referenced in the sequence table are copied from the specified method path to the result set folder.

- The data files, the sequence logbook, and the batch file are copied from the data source directory to the destination directory.

- According to the information in the sequence table, a copy of the corresponding method is copied to each data file as DA.M.

When the result set migration is completed, a success message is displayed in the **Messages and warnings** field. Otherwise a warning message indicates any problem during migration. Details about the warning can be obtained by double-clicking the warning message.

Postsequence Operation

You can specify what happens after a sequence has finished during normal execution or when ChemStation encounters an error during sequence operation. For LC operation, this is done by activating the post-sequence Cmd/Macro check box of the Sequence Parameters where you have a choice of:

- Setting the system to a STANDBY state where the pump and lamp are off,
- setting the system to a LAMPOFF state where all lamps are off (LC and CE only),
- Setting the system to a PUMPOFF state where all pumps are off (LC and CE only), or
- Using a default SHUTDOWN macro or modifying SHUTDOWN.MAC to determine specific operation.

  For example, you may want to switch off your system after your sequence has completed. Other uses of the shutdown macro could be to set your flow to zero or slowly reduce your flow.

In the Sequence Parameters, you may specify any custom macro to run by including its name in the Post-Sequence Cmd/Macro field and checking the box.

Not Ready Timeout (LC and CE only)

The Not Ready Timeout in the Sequence Parameters is the length of time the system will wait for the instrument to become ready — after this time the system will shut down.

Wait Time (LC and CE only)

Sequence Parameters allows you to specify a wait time which is executed after loading method and before injecting with that method. This can be useful for column/capillary re-equilibration when using new analysis conditions.

Automatic Recalibration

Calibration is often done after a change in operating conditions, for example, after changing a column or capillary. Automatic recalibration is usually done at the start of a sequence of analyses or at regular intervals during a sequence as part of a program to compensate for factors that affect the analytical performance.

There are two ways to specify automatic sequence recalibration:

• explicit calibration sequences, or

• cyclic calibration sequences.

Recalibration Using Preference Mode "Unique Folder Creation ON"

While performing a recalibration, the calibration table of the used method is update according the defined method settings. Using the data storage mode "Unique Folder Creation ON", the recalibrated methods are available within the result set. The calibration table of the sequence method is updated during that process. In addition the DA.M method of the individual data files contains the updated calibration used for result creation.

Recalibration Using Preference Mode "Unique Folder Creation OFF"

While performing a recalibration, the calibration table of the used method is update according the defined method settings. Using the data storage mode "Unique Folder Creation OFF", the calibration table of the master method is updated during the recalibration.

Specifying Recalibrations

The recalibration parameters for the sequence are entered directly into the sequence table. These parameters define how the method is recalibrated during a sequence.

Recalibration Parameters in the Sequence Table

The response factor and retention/migration times can be updated in several ways. The calibration level, update response factor and update retention/migration times are instructions used in the data analysis when recalibrating the calibration table.

When Calibration is entered in the SampleType column of the sample table, the following columns become active and can be edited:

- Cal Level
- Update RT
- Update RF
- Interval

The values that can be entered into each of these columns are shown in the table.

Table 9   Recalibration Parameters in Sequence Table

CAL Level	Update RT	Update RF	Interval
Calibration table Level # (1-999)	No update	No update	Cyclic recalibration interval # (1-999)
	Average	Average	Blank
	Replace	Replace	
		Bracket	
		Delta%	

The table shows the columns in the sequence table that contain the recalibration parameters and the values that can be entered.

No Update

Does not change the response factor or retention/migration time.

Replace

Replaces the previous retention/migration times and the response (areas or heights) with those from the current run only. The response is not changed for any peak that is not found in this recalibration run.

Average

Averages the retention/migration times and responses (areas or heights) for each peak based on the original calibration run and all averaged recalibrations since then. If a peak is missing in one of the recalibrations, the average response of the peak will not suffer.

Bracket

The samples are bracketed by pre-sample and post-sample calibrations. Evaluation is done when the last calibration sample of the closing bracket has been run. The existing calibration data is replaced by the result data of the calibration run of the opening bracket. The closing bracket calibrations are averaged to that calibration table.

Interval

The Interval determines how often a calibration is done during a sequence. Calibration frequency corresponds to the number of sample injections that are done before the next set of calibration injections takes place. At the start of the analysis, a calibration is done and the results (response factors) are entered into the calibration table. These results are then used in subsequent quantitative calculations. After the specified number of injections have been done, another calibration is made and the results are entered into the calibration table overwriting the results of the previous calibration run.

Delta%

The delta% calculation allows you to compare response factors from an analysis with response factors entered manually into a calibration table. The delta% is then applied to all of the calibrated peaks in the table. You can identify several internal standards, and their measured response factors are then used to calculate new response factors for the other peaks. You identify which internal standard is to be used for the delta% calculation for each peak in the calibration table.

Types of Sequences

The following are types of sequences:

- explicit calibration sequences,
- explicit single-level calibration sequences,
- cyclic multiple-level calibration sequences,
- explicit and cyclic calibrations together in a sequence, and
- cyclic calibration sequences with bracketed calibrations.

Explicit Calibration Sequences

This type of sequence recalibrates at defined intervals specified by you in the sequence table.

For explicit calibration sequences, the calibration samples are entered in the sequence without an interval entry in the sequence table. A recalibration is done once for each calibration sample entry in the sequence table.

Cyclic Single-Level Calibration Sequences

This type of sequence uses the same vial, that is, the calibration sample at regular intervals in the sequence.

The interval entry in the sequence table determines how the recalibration is done. For example, an interval value of 2 will recalibrate after every two sample vials in the sequence.

Cyclic Multiple-Level Calibration Sequences

This type of sequence uses different calibration samples to recalibrate a multiple-level calibrated method.

The following example describes a two-method sequence comprising method A and method B to analyze two groups of samples. Both methods are multiple-level calibration methods that will recalibrate automatically at defined intervals.

For each method the Sequence Table has three entries:

- Two calibration levels:
    - Sequence lines 1 and 2 in method A.
    - Sequence lines 8 and 9 in method B.
- Five entries for the samples:
    - Sequence line 3 through 7 in method A.
    - Sequence line 10 through 14 for method B.

The calibrations are specified at regular intervals by the recalibration interval entry in the Sequence Recalibration Table.

- Method A will recalibrate after every two samples.
- Method B will recalibrate after every three samples.

The Sequence Table below is truncated to simplify the example.

Table 10   Sequence Table for Method A and Method B

Line	Vial	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval
1	1	Method A	1	Calibration	1	Average	No update	2
2	2	Method A	1	Calibration	2	Average	No update	2
3	10	Method A	1					
4	11	Method A	1					
5	12	Method A	1					
6	13	Method A	1					
7	14	Method A	1					

Table 10   Sequence Table for Method A and Method B

Line	Vial	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval
8	3	Method B	1	Calibration	1	Average	No update	3
9	5	Method B	2	Calibration	2	Average	No update	3
10	20	Method B	1					
11	21	Method B	1					
12	22	Method B	1					
13	23	Method B	1					
14	24	Method B	1					

Method A Analysis Order

Method A is the first part of the two-method sequence.

Table 11   Method A Analysis Order

Inj No.	Method	Vial	Operation
1	Method A	1	Calibration level 1 and report
2	Method A	2	Calibration level 2 and report
3	Method A	10	Sample analysis and report
4	Method A	11	Sample analysis and report
5	Method A	1	Calibration level 1 and report
6	Method A	2	Calibration level 2 and report
7	Method A	12	Sample analysis and report
8	Method A	13	Sample analysis and report
9	Method A	1	Calibration level 1 and report
10	Method A	2	Calibration level 2 and report
11	Method A	14	Sample analysis and report

Method B Analysis Order

Method B is the second part of the two-method sequence. Method B differs from Method A in that there are two injections per vial for calibration level 2. The Interval entry is set to 3.

Table 12 Method B Analysis Order

Inj No.	Method	Vial	Operation
12	Method B	3	Calibration level 1 and report
13	Method B	5	Calibration level 2 and report
14	Method B	5	Calibration level 2 and report
15	Method B	20	Sample analysis and report
16	Method B	21	Sample analysis and report
17	Method B	22	Sample analysis and report
18	Method B	3	Calibration level 1 and report
19	Method B	5	Calibration level 2 and report
20	Method B	5	Calibration level 2 and report
21	Method B	23	Sample analysis and report
22	Method B	24	Sample analysis and report

Note that the results shown in Table 11 on page 113and Table 12 on page 114can be obtained by using Partial Sequence to see a preview of the run order after setting up the Sequence Table.

Explicit and Cyclic Calibrations Together

This type of sequence comprises explicit and cyclic calibrations in the same sequence.

This feature allows you to recalibrate the method completely at the beginning of a sequence (*explicit recalibration*) and then update the calibration (*cyclic recalibration*) during the sequence.

*   *Two calibration lines for each calibration level in the Sequence table must be specified.* One calibration line is for the explicit recalibration entry and the other for the cyclic recalibration entry.

*   The sequence table *must* have entries for each calibration line and all cyclic recalibration vials *must* appear before both the explicit recalibration and sample entries.

Example

The sequence table below illustrates a single-level calibrated method called SimpReg. It is truncated to simplify the example.

Table 13   Sequence Table for SIMPREG

Line	Vial	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval
1	1	SimpReg	1	Calibration	1	Average	Average	3
2	1	SimpReg	1	Calibration	1	Replace	Replace	
3	2	SimpReg	1					
4	3	SimpReg	1					
5	4	SimpReg	1					
6	5	SimpReg	1					
7	6	SimpReg	1					

There are two entries for the single calibration level.

- The first calibration line is for the same level but averages the calibration parameters. The interval entry specifies that the recalibration is done after every three samples.

- The second entry replaces all the recalibration parameters, that is, a complete recalibration is done. It has *no* recalibration interval.

Sequence Table   The sequence table comprises seven lines. The first line specifies the cyclic recalibration sample. The second line specifies the explicit recalibration that is done only once at the beginning of the sequence. The third through seventh lines specify the samples to be analyzed.

The order of the entries into the sequence table is very important. All cyclic recalibration vial entries specifying cyclic calibration *must* appear *before* the sample entries or any explicit recalibration entries for the method.

SimpReg Analysis Order

The table below describes the analysis order for the SimpReg method.

Table 14   SimpReg Analysis Order

Seq Line	Inj No.	Method	Vial	Operation
2	1	SimpReg	1	Simple calibration
1	2	SimpReg	1	Regular calibration
3	3	SimpReg	2	Sample analysis
3	4	SimpReg	3	Sample analysis
4	5	SimpReg	4	Sample analysis
5	6	SimpReg	1	Regular calibration
6	7	SimpReg	5	Sample analysis
7	8	SimpReg	6	Sample analysis

Cyclic Calibration Sequences with Bracketing

In a cyclic calibrated sequence with bracketing, the calibration table used to calculate the unknown quantitative results is generated by averaging the results of the current calibration with those of the previous calibration. This new calibration table is a more accurate representation of the instrument response at the time the sample was analyzed.

Example

Consider the following situation:

- The instrument response is drifting.
- Three injections of an identical two-component mixture are specified.
- Two injections are specified as calibration samples, and one is specified as a sample.
- The first and third are calibration samples.
- The second injection is a sample.

To obtain a precise quantitative result for injection two (the sample), an interpolation must be made between the two calibration samples; see the figure. This process is known as bracketing.
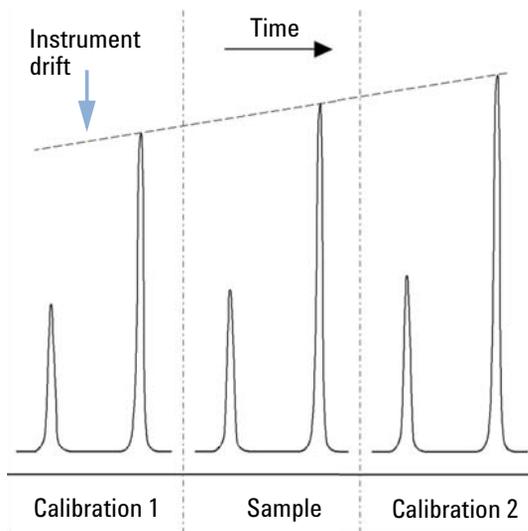

Figure 22   Bracketing

Bracketed Sequence Operation

- The first calibration vials are analyzed.

- The sample vials are analyzed.

- The next calibration vials are analyzed.

- The calibration table is produced by replacing the existing response factors by new ones and averaging the following calibration runs into a new calibration table.

- The sample vial data files are evaluated, and reports are generated.

- The sequence returns to step 2 if there are more sample vials to be analyzed.

Example

This section describes a sequence bracketing example comprising one method called Brack.M. The Brack.M method is a two-level internal standard method using cyclic calibration.

Sequence Table   The sequence table of Brack.M (next page) is truncated to simplify the example. It consists of seven lines. The first two lines define the recalibration conditions for each level. The remaining lines define the samples to be analyzed.

More specifically, the sequence table of Brack.M method has:

- A Bracket entry in the Update Response Factor column that specifies bracketing of samples with calibration samples.

- A Replace entry in the Update Retention/Migration Times column which specifies a replacement of the retention/migration times.

- An entry of 3 in the Recalib Interval column that specifies recalibration after every three samples.

Table 15   Sequence Table for BRACK-M

Line	Vial	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval
1	1	BRACK-M	2	Calibration	1	Bracket	Replace	3
2	2	BRACK-M	2	Calibration	2	Bracket	Replace	3
3	10	BRACK-M	1					
4	11	BRACK-M	1					
5	12	BRACK-M	1					
6	13	BRACK-M	1					
7	14	BRACK-M	1					

```
--------------------------------------------------------------------------------
Run   Method    Vial Inj  DataFile     Lvl  Upd   Upd   Operation
No.   Name      No.  No.   Name         No.  RF    Ret
--------------------------------------------------------------------------------
 1    Brack.M    1    1    c1-03001.d   1    R     R     Report for Calibration Run No.1
 2    Brack.M    1    2    c1-03002.d   1    A     R     Report for Calibration Run No.2
 3    Brack.M    2    1    c2-03001.d   2    R     R     Report for Calibration Run No.3
 4    Brack.M    2    2    c2-03002.d   2    A     R     Report for Calibration Run No.4
                                                         Print Calibration Table
 5    Brack.M   10    1    010-0301.d                    Sample Analysis, no report
 6    Brack.M   11    1    011-0301.d                    Sample Analysis, no report
 7    Brack.M   12    1    012-0301.d                    Sample Analysis, no report
 8    Brack.M    1    1    c1-03003.d   1    A     R     Calibration Analysis, no report
 9    Brack.M    1    2    c1-03004.d   1    A     R     Calibration Analysis, no report
10    Brack.M    2    1    c2-03003.d   2    A     R     Calibration Analysis, no report
11    Brack.M    2    2    c2-03004.d   2    A     R     Calibration Analysis, no report
                                                         Print Calibration Table
                         010-0301.d                    Report for  Sample    Run No.5
                         011-0301.d                    Report for  Sample    Run No.6
                         012-0301.d                    Report for  Sample    Run No.7
                         c1-03003.d   1    R           Report for Calibration Run No.8
                         c1-03004.d   1    A           Report for Calibration Run No.9
                         c2-03003.d   2    R           Report for Calibration Run No.10
                         c2-03004.d   2    A           Report for Calibration Run No.11
12    Brack.M   13    1    013-0301.d                    Sample Analysis, no report
13    Brack.M   14    1    014-0301.d                    Sample Analysis, no report
14    Brack.M    1    1    c1-03005.d   1    A     R     Calibration Analysis, no report
15    Brack.M    1    2    c1-03006.d   1    A     R     Calibration Analysis, no report
16    Brack.M    2    1    c2-03005.d   2    A     R     Calibration Analysis, no report
17    Brack.M    2    2    c2-03006.d   2    A     R     Calibration Analysis, no report
                                                         Print Calibration Table
                         013-0301.d                    Report for Sample Run No.12
                         014-0301.d                    Report for Sample Run No.13
                         c1-03005.d   1    R           Report for Calibration Run No.14
                         c1-03006.d   1    A           Report for Calibration Run No.15
                         c2-03005.d   2    R           Report for Calibration Run No.16
                         c2-03006.d   2    A           Report for Calibration Run No.17
--------------------------------------------------------------------------------
                              Where A = average
                                    R = replace
```

Figure 23   Bracketed Sequence Analysis Order

Cyclic Recalibration Sequences with Multiple Vials Containing the Same Dilution of a Standard

Cyclic Recalibration Sequence with "Round-Robin" Calibration Vial Usage

When running a large sequence that performs cyclic recalibrations, that is, performs an automatic recalibration after a fixed number of sample injections, there is a potential risk of emptying the volume of calibration vial during the course of the sequence. The ChemStation sequence table provides a means of using a series of vials that contain the same dilution of a standard to be used in a *round-robin* fashion.

With this capability, large sequences can be defined with automatic recalibrations at fixed intervals using multiple calibration vials for each level and each calibration vial would be consumed to the same extent.

By defining an appropriate number of calibration vials, it is even possible to guarantee that each calibration vial is only used once. This is an important requirement in cases where a fresh calibration vial is required for every recalibration, for example, because the analyte evaporates once the septum is punctured or starts degrading after getting in contact with the steel needle. The following section describes how the ChemStation sequence table needs to be set up to fulfill these requirements.

Determine the total number of calibration vials for each level based on the estimated calibrant usage throughout the sequence.

Set up a separate cyclic recalibration line for each calibration vial. Lines defined for the same calibration level must be in adjacent sequence lines and the defined vial positions must be adjacent, too. Choose an identical recalibration interval for all calibration lines. For example, if your sequence should recalibrate after every 6 sample injections, set the recalibration interval to 6.

Table 16 Cyclic Recalibration Sequence with 3 Vials Defined for Each Level

Vial No.	Sample Name	Sample Type	Method Name	No. of Inj.	Lvl	Upd RT	Upd RF	Interval
1	Cal1a	Calib	MethodA	1	1	Avg	Avg	6
2	Cal1b	Calib	MethodA	1	1	Avg	Avg	6
3	Cal1c	Calib	MethodA	1	1	Avg	Avg	6
5	Cal2a	Calib	MethodA	1	2	Avg	Avg	6
6	Cal2b	Calib	MethodA	1	2	Avg	Avg	6
7	Cal2c	Calib	MethodA	1	2	Avg	Avg	6
10	Sample10	Sample	MethodA	6				
11	Sample11	Sample	MethodA	6				
12	Sample12	Sample	MethodA	6				
13	Sample13	Sample	MethodA	6				
14	Sample14	Sample	MethodA	6				

The execution order is:

- Vial 1 (Cal1a)
- Vial 5 (Cal2a)
- 6 injections out of vial 10 (Sample10)
- Vial 2 (Cal1b)
- Via 6 (Cal2b)
- 6 injections out of vial 11 (Sample11)
- Vial 3 (Cal1c)
- Vial 7 (Cal2c)
- 6 injections out of vial 12 (Sample12)
- Vial 1 (Cal1a)
- Vial 5 (Cal2a)
- 6 injections out of vial 13 (Sample13)
- Vial 2 (Cal1b)
- Vial 6 (Cal2b)
- etc.

Cyclic Recalibrations Where Each Calibration Uses a Different Vial

To ensure that every calibration vial be injected only once, the sequence must define a sufficient number of different calibration vials, so that the *round-robin* order described in the previous example is not applied. For example, if the sequence processes 80 sample vials with recalibrations required after every 10 samples, the sequence table must contain 80/10 + 1= 9 calibration lines for each level.

As in the previous example, the calibration lines must be adjacent sequence lines that reference adjacent vial positions.

Bracketing Sequence that Uses Different Vials for Opening and Closing Bracket

The same capability is available for bracketing sequences. By defining the appropriate vial range of calibration vials, a bracketing sequence can be defined so that different calibration vials are used for the opening and closing brackets. In this case, too, calibration lines in the sequence must be adjacent just like the vial positions of the calibration vials.

Whether or not the bracketing calibration vials are used in round-robin mode or for one single injection only simply depends on the total number of calibration vials for each level and the number of recalibrations required by the sequence.

The following example defines 3 sample injections that are bracketed by calibrations. The opening bracket uses a different calibration vial than the closing bracket. Recalibrations are required after every sample injection. so the recalibration interval has to be 1. The number of calibration lines per level is the number of samples plus one.

Table 17   Different Vials Used for Opening and Closing Brackets

Vial No.	Sample Name	Sample Type	Method Name	No. of Inj.	Lvl	Upd RT	Upd RF	Interval
1	Cal1a	Calib	MethodA	1	1	Brkt	Brkt	1
2	Cal1b	Calib	MethodA	1	1	Brkt	Brkt	1
3	Cal1c	Calib	MethodA	1	1	Brkt	Brkt	1
4	Cal1d	Calib	MethodA	1	1	Brkt	Brkt	1
10	Sample10	Sample	MethodA	1				
11	Sample11	Sample	MethodA	1				
12	Sample12	Sample	MethodA	1				

The execution order for this sequence is:

- Vial 1 (Cal1a), Opening Bracket 1
- Vial 10 (Sample10)
- Vial 2 (Cal1b), Closing Bracket 1 and Opening Bracket 2
- Vial 11 (Sample11)
- Vial 3 (Cal1c), Closing Bracket 2 and Opening Bracket 3
- Vial 12 (Sample 12)
- Vial 4 (Cal1d), Closing Bracket 3
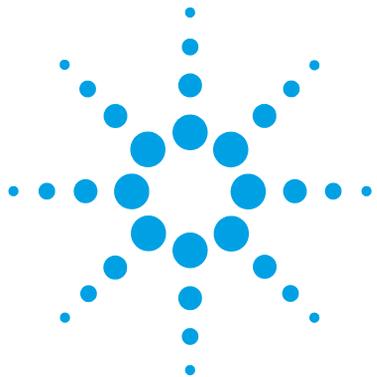
5
Run Queue and Queue Planner

This chapter explains the concepts of Run Queue and Queue Planner. It explains how to add single samples, sequences, or pauses to the Run Queue.

Agilent Technologies

Supported Workflows

The Run Queue controls all runs in ChemStation:

- Whenever you use the **RunControl > Run Method** or **RunControl > Run Sequence** command to run a method or a sequence, the item is first added to the Run Queue and is then automatically started from there. If the Run Queue is currently paused, the item will be added to the front of the queue, followed by a queueable pause. That way, the instrument will return to the paused status when the run has been finished.

- The Run Queue also allows you to schedule a series of samples and sequences along with additional parameters. You can add samples or sequences to the queue with the **RunControl > Queue Method…** or **RunControl > Queue Sequence…** command. With the Run Queue, you can automate lengthy operations such as overnight or weekend jobs. In addition to the samples and sequences, you can also schedule pauses. In these pauses, ChemStation shows a customizable message and waits for a user confirmation.

With the Queue Planner, you can prepare Queue Plans in advance and add them to the Run Queue at a later point in time.

The following workflows are supported:

- Run a single sample
- Run a single sequence
- Queue a singe sample
- Queue a single sequence
    - **a** Select a classic ChemStation sequence template or an Easy Sequence template
    - **b** Edit or review the sequence table
    - **c** Edit or review the sequence parameters
    - **d** Save the settings
    - **e** Add the sequence to the queue
- Change the Run Queue

- Prepare a Queue Plan
- Add a predefined set of sequences to the Run Queue
    a Select a Queue Plan
    b Add the plan to the Run Queue

In the History Queue, you can always see which runs have been executed on the current instrument.

Run Queue and Queue Planner are only available in online ChemStation sessions in the **Method and Run Control** view.

Using the Run Queue

The Run Queue is both in the **Instrument Control** tab or in the **Run Queue** tab. In the Instrument Control tab, you can show or hide the Run Queue with the **View > Run Queue** command.
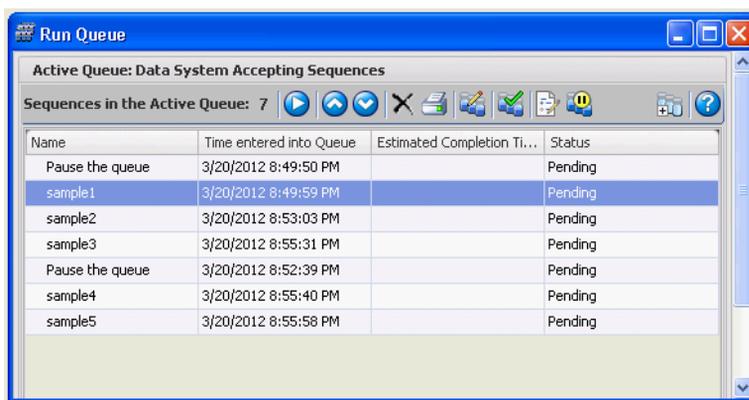
Figure 24 **Run Queue** dialog

You can add an item to the front or to the back of the queue. As long as the status of the items in the queue is pending, you can change both the execution order and the properties of the item. Depending on the Active Queue Options, the first item in the queue either starts when the data system is ready or when you resume the queue.

The Run Queue supports single samples, Easy Sequence templates, and classic ChemStation sequences. The only items that cannot be added to the Run Queue are Partial Sequences, Priority Samples, and Runs that are started directly from the instrument.

For more information on Easy Sequence, see the online help system. **Easy Sequence Setup** tutorials are available in online help.

Single Samples in the Run Queue

To add a single sample to the queue, use the menu **RunControl > Queue Method...**. You can modify all parameters in the **Queue Method** dialog.

Sequences in the Run Queue

To add a sequence to the queue, use the menu **RunControl > Queue Sequence...**. You can modify the sequence table and sequence parameters without changing the currently loaded sequence. Before finally queuing the sequence, a dialog offers you to either add the sequence to the queue or to save it as a new sequence template.

The **Finish Queue Sequence** dialog also contains the check box **Delete temporary Sequence Template after completion**. ChemStation always keeps a copy of the queued sequence template in a temporary directory. This temporary sequence template will be used to run the sequence from the queue. As the same sequence may be queued several times using different parameters, ChemStation needs a separate copy for each queued item.

Depending on the status of the check box, this temporary sequence template will be kept or deleted when the queue continues with the next item. The check box may be selected or cleared by default, depending on the settings for **Unique Folder Creation** (see "Preferences - Sequence Tab" on page 97):

- With **Unique Folder Creation OFF**:

  The **Delete temporary Sequence Template after completion** check box is cleared by default.

  If you want to reprocess the data, you will need the sequence template, therefore we recommend to keep a copy of this file. By default, it is saved under Chem32\<instrument>\SEQUENCE.

- With **Unique Folder Creation ON**:

  The **Delete temporary Sequence Template after completion** check box is selected by default.

  All information required for reprocessing is already available in the result set, therefore you do not need a copy of the temporary sequence template. However, if you select the check box, a copy is saved by default under **Chem32\<instrument>\TEMP\AESEQ**.

Pauses in the Run Queue

To schedule a pause to the queue, click **Add Pause to Queue** in the Run Queue toolbar. In these pauses, ChemStation shows a customizable message and waits for a user confirmation.

Using the Queue Planner

With the Queue Planner, you can prepare an ordered set of sequences (Easy Sequence template *.es or classic ChemStation sequence templates *.s), or pauses. The entire Queue Plan can be added either to the back or to the front of the Run Queue.

The queue plan is saved as a *.qpl file. You open the Queue Planner in the **Method and Run Control** view via the menu **RunControl > Queue Planner.**...
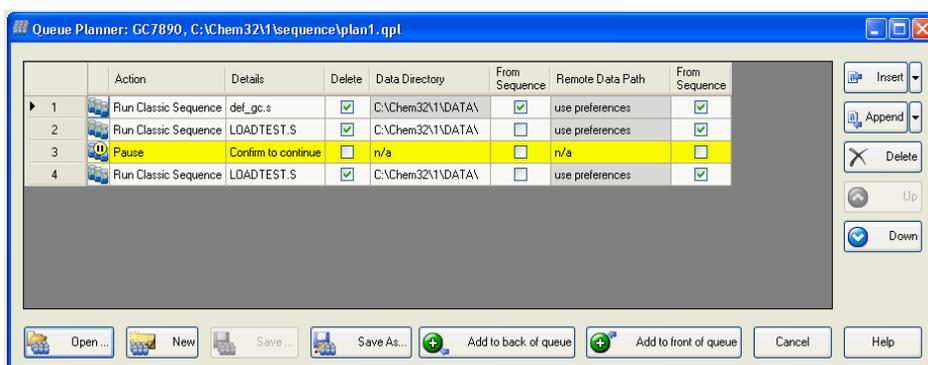
Figure 25   Queue Planner

As in the Run Queue, when you add a pause, you can provide your own message in the **Details** column. When the Sequence Queue reaches the pause, ChemStation stops, and displays the given message. A user must confirm the message before the queue can continue.

For more information on the user interface, see the online help system.

6
Data Analysis and Review Concepts

This chapter outlines the data analysis and data review options. In OpenLAB CDS ChemStation Edition, these options are available in two separate views.

Data Analysis

Once the data has been acquired, it can be analyzed in **ChemStation Data Analysis** view. When selecting the **Data** tab of the ChemStation Explorer, you can load all the runs of a sequence or all single runs in a specific folder by double-clicking the corresponding symbol. The corresponding data set is then available in the Navigation Table.
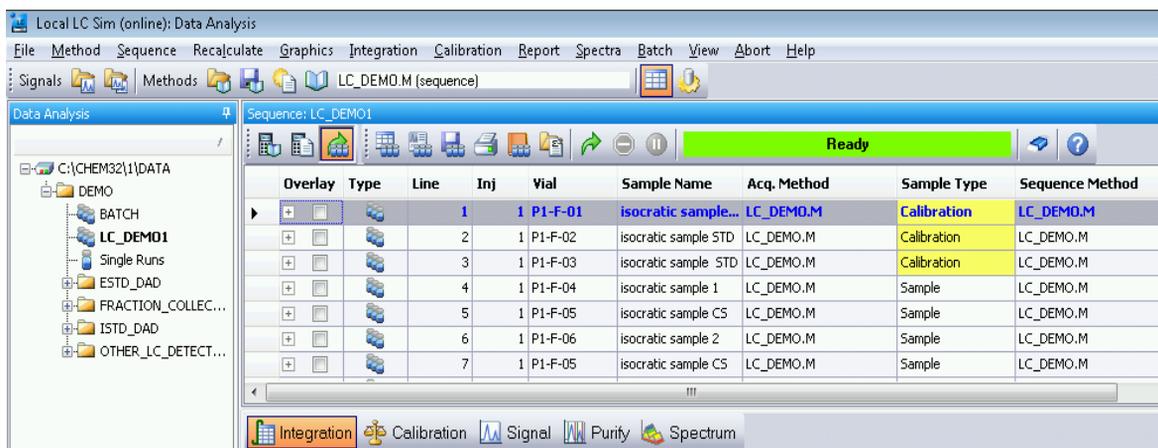
Figure 26   Loading a sequence from the ChemStation Explorer into the Navigation Table

The main body of the Navigation Table consists of a list of all runs of the set. You can load a run into ChemStation memory by double-clicking the relevant line in the Navigation Table. Additionally, right-clicking on a run offers several options, for example, to load or overlay specific signals from the file, to export the data, or to view the acquisition method parameters.

Sequence runs are (in the Reprocessing mode) loaded with the sequence method that has been used during acquisition or reprocessing. The name of this method is shown in the toolbar, and also in the **Sequence Method** column in the Navigation Table. The acquisition method name is shown in the **Acq Method** column.

Single runs may be loaded with one of the following methods, depending on the settings under **Preferences > Signal/Review options** (see figure below):

*   If the check box (1) is selected, and the single run was last analyzed or reported with a method located in one of the currently defined master method paths, the single run is loaded with this master method. The method is shown in the **Analysis Method** column in the Navigation Table.

*   If the check box (1) is cleared, single runs are loaded with the master method that was last loaded in ChemStation.

The acquisition method name is shown in the **Acq Method** column.

ChemStation allows you to specify default actions that are performed automatically when a data file is loaded from the Navigation Table. These include data analysis tasks like integrating the chromatogram directly after loading, or printing a report for each single injection (see figure below).
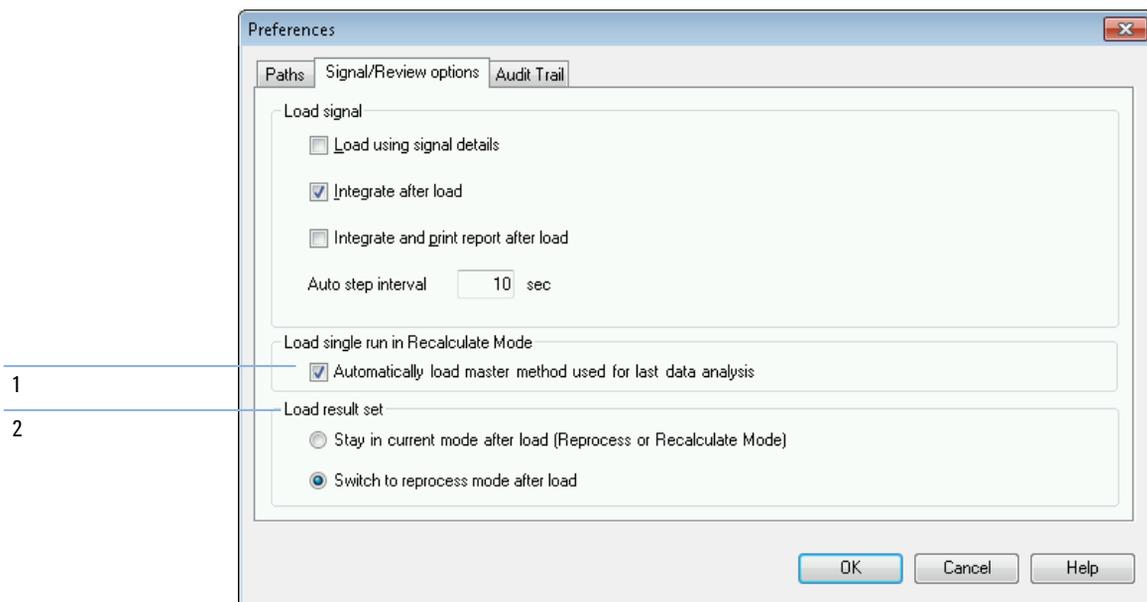
Figure 27   **Signal/Review options** tab of the **Preferences** dialog

Data Analysis Modes

You can choose between the following Data Analysis modes:

- Recalculation mode
- Last Result mode
- Reprocessing mode

These modes are accessible either via the **View** menu or via the tool set (see figure below).

Figure 28   Mode selection

For each of the modes, the tool set contains specific functions. The modes and the respective functions are described in the following sections. In the **Signal/Review Options** tab of the **Preferences** dialog box, you can choose which mode should be active by default when you load a result set (see Figure 27 on page 135, marker 2).

Recalculation Mode

Once the run is loaded, you can review it, that is, adjust data analysis parameters, integrate the signals and finally print a report. In this case you analyze the run as a single run without taking the sequence context into account or using the features of the sequence table. The Navigation Table for this type of data analysis provides the toolset shown in the figure below.

Figure 29 Recalculation toolset of the Navigation Table

With this toolset, you can jump to the beginning or end of the navigation table, step on to the next or previous run, automatically step through the runs, stop automatic stepping, recalculate a run using a specific method, or clear the Navigation Table.

Recalculation means analyzing on a run-per-run base. Only the runs shown in the Navigation Table are analyzed. If you applied a filter to the Navigation Table, only those runs actually shown in the table are recalculated. The sorting of the Navigation Table is also taken into account.

You can use recalculation in the following workflows, for example:

• You want to review the data files of a result set with a different method that is not currently in the result set, for example, a master method not used for acquisition, because your workflow employs separate acquisition and data analysis methods.

• You have edited a sequence method and want to review only specific runs using this method in order to check how well these parameters apply for different runs.

Data Analysis Method for Single Runs

In previous releases, no method was automatically loaded along with any data file in Recalculate mode. From release C.01.05, you can select the check box to automatically load the master method used for the last data analysis (see Figure 27 on page 135, marker 1). If this check box is selected, single runs are loaded with the corresponding master method, if the method still exists at the given location.

Recalculate With Specific Method

With this function, you can recalculate the runs shown in the Navigation Table using a specific master method. You specify the required master method in the **Recalculate With Method** dialog (see figure below). If the selected master method uses Intelligent Reporting (see "Reporting" on page 169), you can also specify the report template that will be used for the single injection reports.
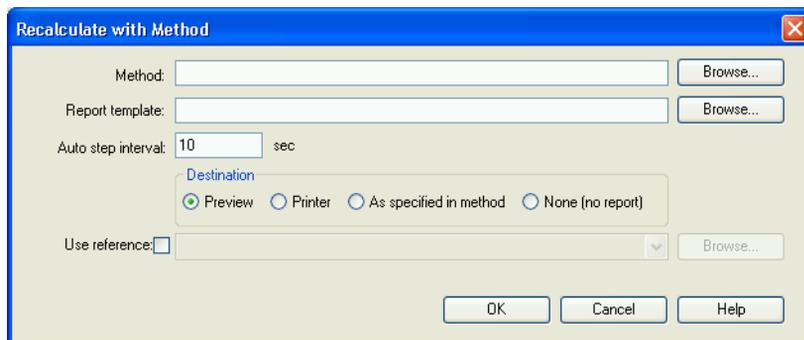
Figure 30 **Recalculate With Method** dialog

The **Browse for methods in master paths** dialog and **Browse for templates in master paths** dialog offer all file locations that you specified in the Preferences.

NOTE	In previous revisions of ChemStation you have been able to recalculate with a specific method by choosing **Use current method**, **Use method from data file**, or **Use sequence method** in the toolbar.

If you select the **Use reference** check box, you can select a data file containing a reference signal. ChemStation uses this signal to calculate the signal-to-noise ratio as defined by European Pharmacopeia. The drop-down list offers the data files that you used during the current session. With the **Browse** button, you can select any data file that is also present in the Navigation Table. If want to use a different reference file, you must first add it to the Navigation Table.

The new reference overwrites the previous one and will be used from now on in signal-to-noise calculations in every report. If you select the **Use reference** check box, but do not select any file, the references are cleared for all recalculated data files, and no signal-to-noise values will be calculated from now on.

Each time you recalculate a sample or generate a report, the data file method (DA.M) is automatically updated with the data analysis parameters of the used method. The path to the specific method is saved as a reference in the data file.

Last Result Mode

Figure 31 Last Result Mode toolset of the Navigation Table

In this mode, the data file method (DA.M) for each run is loaded. DA.M is an exact copy of the method which was used for the last data analysis (during acquisition, reprocessing, or recalculation). So even if the sequence method has been changed in the meantime, you can reproduce the last result with the method originally used. In that way you can, for example, track changes done to the method in successive recalibration steps in a result set.

The method name in the toolbar shows DA.M, which indicates that the data file method is loaded. When you move the mouse pointer over this field, a tool tip additionally displays the full path and name of the method. In addition, the name of the method last used for data analysis (which was copied to DA.M) is shown in the **Analysis Method** column in the Navigation Table. The tool tip of this column shows the full path of this method.

NOTE The DA.M is normally read-only. You cannot manually load it, it is only loaded by ChemStation in the Last Result Mode for recalculation. You can edit it, but you cannot manually save it. If you have edited and changed the method, you cannot generate a report any more, as this would imply saving the DA.M method and thus possibly lead to inconsistencies in the system.

In Last Result Mode, you can update the loaded master method or any master method with the current data analysis parameters from the DA.M, or save a changed DA.M method as an entirely new master method. For example, you loaded a data set analyzed a few weeks or months ago, and you find the data analysis parameters stored in the DA.M useful for your current work. You can then transfer the settings to a master method of your choice. For more information, refer to "Administrating Methods" on page 48.

Reprocessing Mode

A different way to analyze your data is to **Reprocess** a complete sequence. In contrast to recalculation, all runs are reanalyzed in the sequence context, i.e. the calibration tables of the sequence methods are updated in the case of calibration runs, and multipliers, amounts etc. can be changed in the sequence table.

The result set includes all files needed for reprocessing: the data files, a copy of the sequence file, all the sequence methods and all the report templates originally employed with the acquisition. Thus, in order to reprocess a sequence you simply have to load it into the Navigation Table and select the reprocessing tool set.

If it becomes necessary to propagate changes in the sequence method to the corresponding master method as input for all future acquisition runs, you can achieve this conveniently by using the **Update Master Method** functionality (see "Updating DA Parameters in the Master Method" on page 52).

The DA.M is automatically updated each time you reprocess a data file.

For reprocessing, the navigation table provides the following toolset:

Figure 32   Sequence Reprocessing Toolset of the Navigation Table

With this toolset, you can edit the sequence table, edit the sequence parameters, save the current sequence, print the current sequence, show or hide the sequence logbook, view the saved sequence summary report file(s), start sequence reprocessing, stop the sequence, or pause the sequence.

Note that the reprocessing icons in the Navigation Table are available only for result sets generated with ChemStation B.02.01 and higher. Reprocessing in **Data Analysis** is not accessible for single run data, for data generated prior to B.02.01, and for data acquired while **Unique Folder Creation** is switched off (see "Preferences - Sequence Tab" on page 97). Such sequences need to be reprocessed in **Method and Run Control**, defining the sequence parameter **Part of method to run** as **Reprocess Only**. For sequences generated with ChemStation B.02.01 and higher, the reprocessing option in **Method and Run Control** has been removed, and the navigation table offers reprocessing as a **Data Analysis Task**.

Another alternative is to add such samples or sequences to a new self-assembled result set. There you also assign sequence methods, and you can reprocess the entire sequence afterwards (see "Self-Assembled Result Set" on page 144).

Note the following rules with regard to reprocessing:

- When loading a result set into the Navigation Table, ChemStation also automatically loads the sequence file (*.S) that is located in this result set. This sequence file contains all the sequence lines that are related to any data file belonging to this result set.

- All actions are performed on the sequence methods. If changed analysis parameters are to be applied, you have to change the sequence methods.

- During reprocessing, the Batch (*.b) file, the sequence/single run log (*.log), and the Navigation Table are updated. The individual data analysis method (DA.M) of each processed data file is overwritten with the sequence method.

- To add new methods from one of the master method directories to the sequence table, use the ChemStation Explorer to copy the master method to the result set, or click **Method > Update Methods...**. You can then select the new sequence method in the sequence table. It is not possible to add or remove lines in the sequence table.

- In the Sequence Parameters dialog, only the sequence comment and the usage of sequence table information can be changed. All other fields have to be set during data acquisition or do not apply to reprocessing.
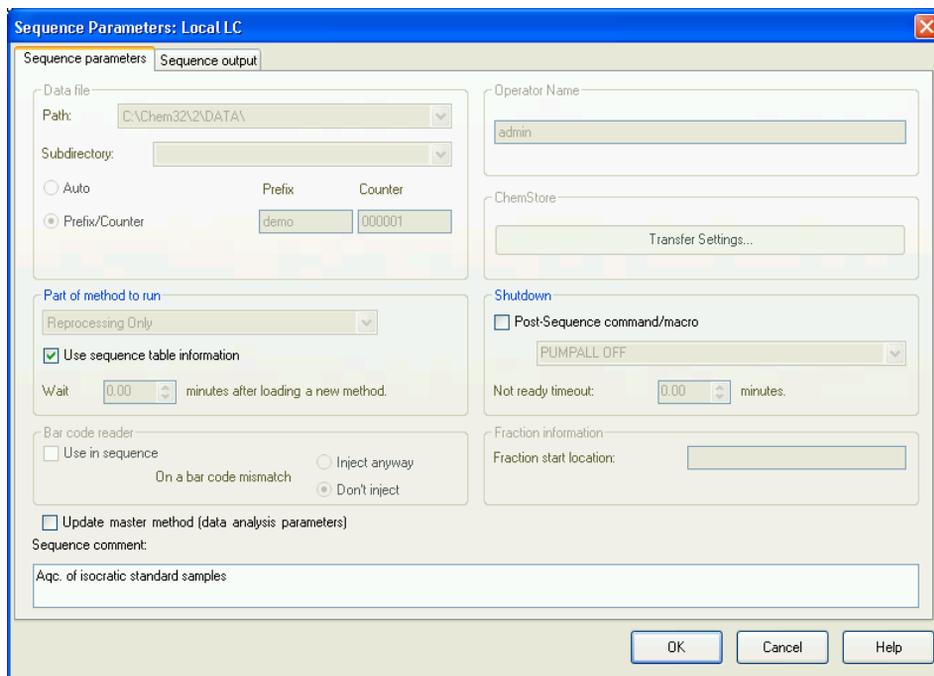
Figure 33 Sequence Parameters in Data Analysis

Treatment of Manual Integration Events

Manual integration events, for example, a manually drawn baseline, are even more data file specific than timed integration events. In case of complicated chromatograms, it is highly desirable to be able to use these events for reprocessing.

Therefore, in ChemStation B.04.01 and higher, manual integration events can be stored directly in the data file instead of the method. Whenever the data file is reviewed or reprocessed, the manual events in the data file are automatically applied. A run containing manual integration events is marked in the Navigation Table in the corresponding column.

In addition to the tools for drawing a baseline and deleting a peak manually, three other tools are available in the user interface to:

- save manual events of the currently shown chromatograms into the data file,

- remove all events from the currently shown chromatograms

- undo the last manual integration events (this is available until the event is saved)

When continuing to the next data file during review in the Navigation Table, ChemStation will check for unsaved manual integration events and ask the user whether he wants to save the events.

Manual events stored in the data file during review in the Navigation Table do not interfere with manual integration events stored during review in the **Batch** mode. These two ways of review are completely separated with regard to the manual events of a data file.

In ChemStation revisions prior to B.04.01, manual integration events could only be stored in the method. In B.04.01, this workflow can still be used. The **Integration** menu in **Data Analysis** view provides the following items in order to handle manual integration events with the method:

Update Manual Events of Method: Save newly drawn manual events to the method.

Apply Manual Events from Method: Apply the manual events currently saved in the method to the currently loaded data file.

Remove Manual Events from Method: Delete the manual events from the method.

In order to convert manual events stored in a method to storage in the data file, apply the events from the method and store the results in the data file. If required, remove the events from the method.

If the **Manual Events** checkbox of the **Integration Events Table** of a method is selected, the manual events of the method are always applied when loading a data file using this method. If the data file contains additional manual events, the events in the data file are used. When the **Manual Events** checkbox is selected, the user is never asked to save the events to the data file.

Self-Assembled Result Set

In the **Data Analysis** view, the Navigation Table shows the contents of the single run or sequence that is loaded. You can load, unload, or add data files to the Navigation Table. Using the **Sequence > Create New Result Set** command, you can create a new self-assembled result set from the data currently shown in the Navigation Table (see "To assemble a new result set" on page 93). Self-assembled result sets can be reprocessed the same way as automatically created result sets.

Unload Current Dataset

Using the **Unload Current Dataset** command from the context menu of the Navigation Table, you can revert the Navigation Table to the original empty state, as it is directly after launching ChemStation. If there is unsaved data, you are asked to save it.

Delete Selected Data File

Using the **Remove selected Data Files** command from the context menu of the Navigation Table, you can remove the selected lines from the Navigation Table. This only removes the reference in the Navigation Table, it does not delete the physical data file from the loaded result set or single run on the file system. It is only possible to delete the references of added/overlaid files.

Updating Methods

In the **Data Analysis** view, you have several options to copy methods between the master method directories and the result sets. For more information, refer to "Administrating Methods" on page 48.

Report Viewer for Data Analysis

Depending on the configuration, ChemStation automatically saves the single injection reports and sequence summary reports to the file system at a certain point in time. With the Report Viewer, you can easily view the saved report files in order to check the results from data acquisition, reprocessing or recalculation.
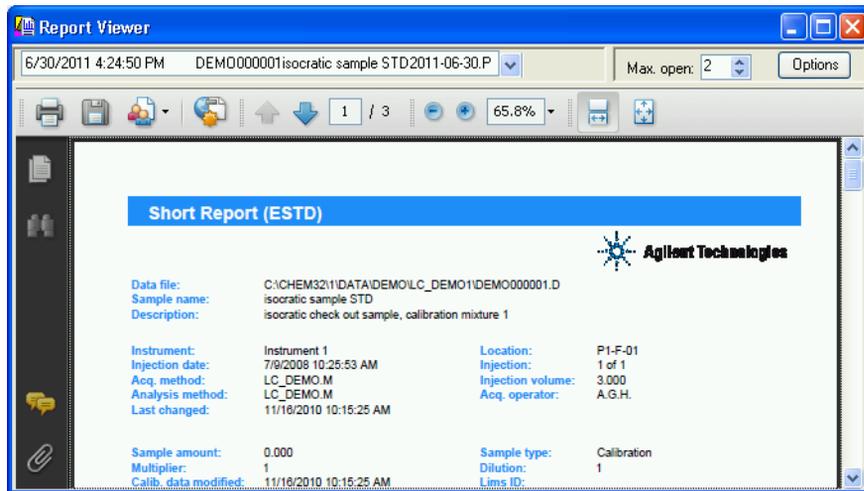
Figure 34   Report Viewer

Using the Report Viewer has the following advantages:

- You can directly open the report files from ChemStation. You do not need to search for the files in the file system.

- Each report opens in a separate floating window. Therefore, you can easily compare different reports by placing the windows side by side.

- You can use the full screen to view the report file.

- You can use the features from Adobe Reader to view .pdf reports.

- You can search for specific text in .txt reports as well as .pdf reports.

- When you reprocess a sequence, you do not have to wait until the entire sequence reprocessing is completed. You can open the saved report files for those sequence samples that are already completed.

Starting the Report Viewer

You can open the Report Viewer via the menu, via toolbar icons, or via the context menu of the Navigation Table. There are different items for sequence summary reports and single injection reports.

To view single injection reports:

- Select the menu **Report > View Report File** to view the report file or files of the loaded signal.

- Select the **View Saved Report File(s)** command from the context menu of a specific sample in the Navigation Table. With this command you can to load the report file or files of any signal, even if it is currently not loaded.

- Click the **View saved Report File(s)** icon from the Workspace Toolbar to view the report file or files of the loaded signal.

To view sequence summary reports:

- Select the menu **Sequence > View Summary Report File**.

- Click the **View Saved Sequence Summary Report File(s)** icon from the Navigation Toolbar (in reprocessing mode).

Configuring the Report Viewer Windows

You can configure several aspects of the Report Viewer behavior. All of these settings are accessible via the **Options** button in the Report Viewer window.

You can define the maximum number of Report Viewer windows that will be open in parallel. The windows are cyclically reused. When you view more report files than the maximum number of Report Viewer windows, the first opened windows are the first to change their content.

NOTE If you do not need to compare multiple reports, we recommend to limit the number of Report Viewer windows to 1.

For comparing multiple reports, it may also be helpful to adjust the title bar of the Report Viewer windows. Various tokens are available for Report Viewer windows displaying sequence summary reports, single injection reports for sequence samples, or single injection reports for single runs. These tokens help you to distinguish the single Report Viewer windows.

The Report Viewer windows are always displayed on top of the ChemStation application. In order to work with ChemStation and the Report Viewer at the same time, you can resize and position both windows in such a way that you can see them both. When you close ChemStation, the windows' size and positions are saved. When you launch ChemStation the next time, the same settings will be used again.

Working with the Report Viewer

You can use the Report Viewer, for example, in the following workflows:

- You set up the method and the sequence to save PDF reports to the file system. After completing the sequence run, you open the report files (sequence summary report or single injection reports) directly from the ChemStation in the Report Viewer. Use the Adobe Reader features such as zooming or searching to check the report in detail.

- You download a sequence that already contains report files from central data storage.

  - To look at the final result, you select the relevant sample in the Navigation Table and open the report file directly from the ChemStation in the Report Viewer.

  - If required, you can change the method and reprocess the sequence. While the reprocessing is ongoing, you start viewing the reports of the already completed samples.

    In the Report Viewer, you can select both old an new report from the list at the top left corner. You can distinguish the reports by their creation date, which is shown as a part of the list entry. Depending on the transfer settings, the data - including the new report files - may be automatically uploaded to central data storage after reprocessing has finished.

- You run a sequence that saves only TXT report files. You can check these report files as well in the Report Viewer.

- You review different reports on the same sequence samples, based on different report styles or templates.

  First, you create a sequence with an extended performance report. You run or reprocess the sequence to obtain the report file. If you are satisfied with the results displayed in this report, you change the sequence method to create a shorter report (for example, select a different report template or the classic report style **Short**). Then you reprocess the sequence to obtain the shorter reports. When you view a report with the Report Viewer, you can switch between the two different reports by selecting them from the list in the top left corner. The creation date of each file is shown as part of the list entry.

Review

In Agilent OpenLAB CDS ChemStation Edition, a new view is available that covers the pure data review workflows of Data Analysis. In this **Review** view, you can generate reports for either an entire sequence, a subset of a sequence, or any selection of data files from different sequences or single samples.

In the **Review** view, you do not load any methods and you do not create any new results as in recalculation or reprocessing. The reports that you generate in the **Review** view only display the results that have already been calculated.

You can select a report template and apply it to specific selection of data files. The combination of template and data file selection determines the output of the generated report.

NOTE	The **Review** view is only available if you activated Intelligent Reporting in the Instrument Configuration in the OpenLAB Control Panel.

Requirements for Intelligent Reporting

ChemStation C.01.05 generates the result data in a specific format (*.ACAML) that is used by Intelligent Reporting. If you want to create reports for data acquired with a ChemStation Version A or B, you must first regenerate results using ChemStation C.01.05 (for example, recalculate the data or generate single injection reports in the Data Analysis view). If the results are not available in the required format, the generated reports in the Review view will not contain any data.

Data File Selection

You can select the required data files by loading the sequences or single runs from the Navigation Tree in the ChemStation Explorer. All available data files are then shown in the Navigation Table. In the Navigation table, you select the specific data files for which you want to see the results in the report.

Loading Data Files

You can load all data files from an entire sequence or Single Runs folder. In the **Data** tab of the ChemStation Explorer, you can either double-click the sequence or use the **Load** command from the context menu to load all included data files.

When you load the data files, the Navigation Table is automatically cleared before the new data files are shown. You can therefore prepare the data for either a *Single Sample Report* or for a *Sequence Summary Report*.

Adding Data Files

If you want to compare the results from different sequences, you can first load one sequence, and then add the required data files from another sequence. In the **Data** tab of the ChemStation Explorer, use the **Add Data Files...** command from the context menu to add only specific data files to the selection already loaded. A dialog opens in which you can select the required data files.

When you add data files, the Navigation Table appends the data files to the list of data files already loaded. You can therefore prepare the data, for example, for *Cross-Sequence Reports*.

Selecting Data Files for Reporting

The Navigation Table shows all data files from the sequence or single sample collection that you double-clicked in the ChemStation Explorer. In the Navigation Table, you select those data files for which you want to create the report. Only the selected lines will be included when you generate a report.

Report Template Selection

You can select the required report templates from the **Report Templates** tab in the ChemStation Explorer. The Navigation Tree shows all report templates in the chem32/repstyle directory.

Report Preview

The resulting report is always determined by both the data selection and the report template. Therefore, ChemStation generates the corresponding report and displays the report preview once you have selected one or more data files and loaded a report template.

You can send the report to a printer or save it as a file (PDF, XLS, DOC, or TXT). If you use a central data storage system, you can also upload the report directly to the central repository.

Possible Review Workflows

You can use the **Review** view for example in the following workflows:

- You load a sequence and select all data files of the sequence. You load a report template and generate a *sequence summary report*.

- After generating a sequence summary report, you load a different report template. You review the same data using a *different report layout*.

- You load a sequence and select only a subset of data files. You load a report template and generate a sequence summary report for *only part of the sequence*.

- After loading a subset of data files, you add other data files (from a sequence or from a collection of single samples). You load a report template and generate a *cross-sample or cross-sequence report*.

7

Calibration

This chapter explains the concepts of calibration.

Agilent Technologies

Definition of Terms

Calibration   Calibration is the process of determining response factors used to calculate absolute component concentrations by injecting specially prepared calibration samples. The calibration table is also used for identification.

Compound   A chemical compound can comprise several peaks, in a multiple signal calibration, typically one per signal. In a single signal calibration a compound refers to one peak.

Calibration Level   A calibration level comprises the calibration points for one calibration sample concentration. In a multisignal calibration the calibration points can be distributed over several signals.

Calibration Point   A calibration point refers to an amount/response ratio for a peak on the calibration curve.

Calibration Sample   A calibration sample, also referred to as a calibration standard or a standard mixture, is a sample containing a known amount of the compound to quantify. In the software the calibration sample is referred to as an injection from the calibration sample vial.

Calibration samples may be purchased from chemical suppliers or they may be prepared using an accurately measured amount of the pure compound. The amount of the compound in the calibration sample is usually expressed as a concentration, typically in ng/µl units.

Types of Calibration

ChemStation offers two types of calibrations, single-level and multilevel calibrations.

Single-Level Calibration

The calibration curve shown in Figure 35 on page 155 contains one point, that is, one level. For the single-level calibration curve, the response of the detector is assumed to be linear over the working range of concentrations for the samples of interest. The response factor for a given component peak is given by the inverse of the slope of the calibration curve line through the point and the origin. A disadvantage of single-level calibration is that the detector response to the sample concentration is assumed to be linear and pass through the origin on a concentration versus response plot. This is not always true and can lead to inaccurate results.
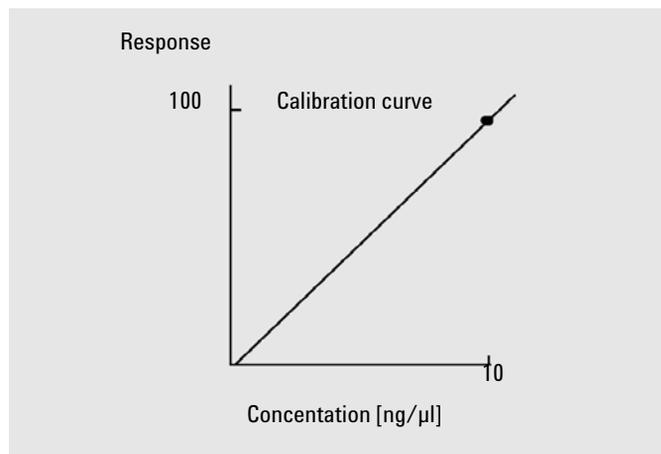
Figure 35   Single-level calibration curve

To obtain accurate quantitative results, a calibration curve should have at least two levels. These levels should bracket the amounts expected to be found in the unknown samples.
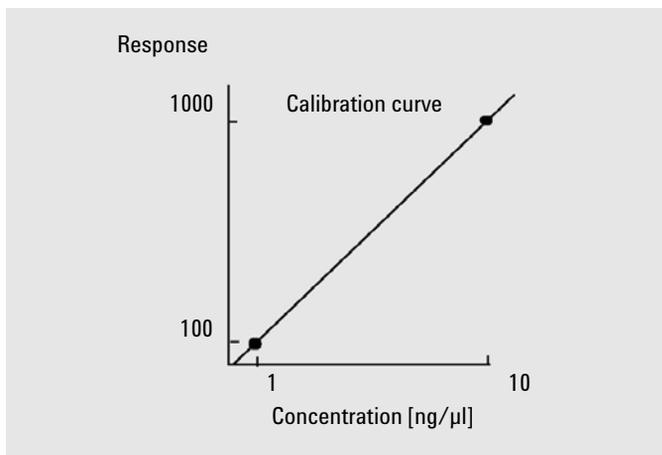
Figure 36   Two-level calibration curve

For example, if you want to quantify a compound, and the unknown samples are expected to range from 1 – 10 ng/μl, then a calibration curve should have at least the two levels as shown in Figure 36 on page 156.

Amount Limits

ChemStation allows you to define the valid quantification ranges in terms of absolute amounts for each component.

Multilevel Calibration

Multilevel calibration can be used when it is not sufficiently accurate to assume that a component shows a linear response or to confirm linearity of the calibration range. Each calibration level corresponds to a calibration sample with a particular concentration of components. Calibration samples should be prepared so that the concentration of each component varies across the range of concentrations expected in the unknown samples. In this way it is possible to allow for a change in detector response with concentration and calculate response factors accordingly.

This multilevel calibration curve has three levels and shows a linear fit through the origin. This method of linear fit through the origin is similar to the single-point method calibration. The detector response to concentration is

assumed to be linear. The difference between the two calibration types is that, with linear fit, the slope of the detector response can be determined by a best fit through a number of points, one for each level.
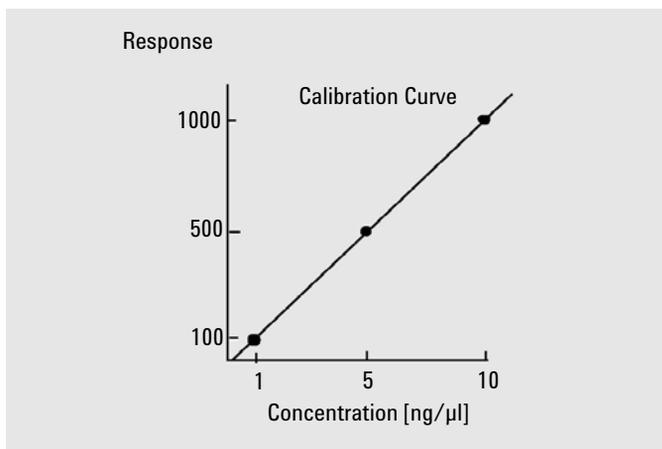
Figure 37   Multi-level calibration curve with three levels

The corresponding calibration table, which is the tabulation of the information used to generate this curve, might look similar to the one shown in Table 18 on page 157.

Table 18   Calibration Table

Level	Amount (ng/µl)	Response (area counts)
1	1	100
2	5	500
3	10	1000

In this example, the calibration samples that were used to generate the three levels had been identified as 1, 2 and 3.

Calibration Ranges

Each multilevel calibration is valid over the range of concentrations used in the calibration samples. Extrapolation of a calibration curve, especially if it is non-linear, is at best an approximation. The valid calibration range for each compound may be defined in the **Compound Details** dialog box. Each entry for that compound can be expressed as lower and upper limits. If these limits are exceeded, the report is annotated.

Calibration Curve Fits

Various curve-fit calculations are available for use with multilevel calibration.

• Piecewise Linear

• Linear

• Log

• Power

• Exponent

• Quadratic

• Cubic

• Average (Response/Amount)

Non-Linear Fit

In some cases, the detector response to changes in sample concentration is not linear. For these types of analyses, a linear regression calibration method is not appropriate and a multilevel calibration calculation should be used.

Origin Treatment

There are four ways to treat the origin when the response curve is plotted:

- ignore the origin,
- include the origin,
- force the origin, or
- connect the origin.

To force the origin to be included in the calibration curve the calibration points are mirrored about the origin from the first quadrant into the third quadrant. Using all points for the regression calculation ensures that the resulting calibration curve passes through the origin. This is also explained in Figure 38 on page 159.
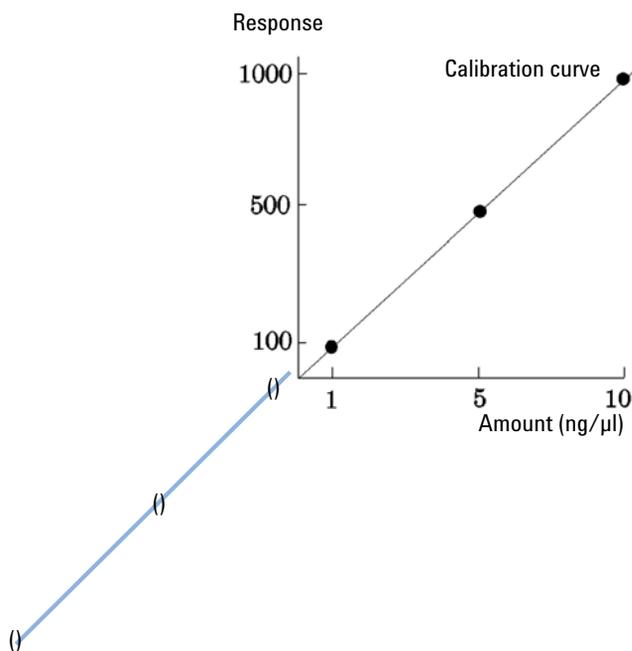
Figure 38   Forcing the origin to be included

For further information about calibration curve fits and origin treatment, see the *online help* file.

Calibration Point Weighting

When setting up your default calibration curve, you can specify the relative weighting (or importance) of the various calibration points used to generate the curve.

The following weighting options can be selected:

Weight	Description
Equal	All calibration points have equal weight in the curve.
Linear (Amnt)	A calibration point with the amount x has the weighting $1/x$ normalized to the smallest amount so that the largest weight factor is 1. Normalization is done by multiplying the weight with the smallest amount. For example the weight of a calibration point with the amount x is $(1/x) \times a$ where a is the smallest amount of the calibrated compound prepared in the calibration standards. If the origin is included it is assigned the mean of the weightings of the other calibration points.
Linear (Resp)	A calibration point with the response y has the weighting $1/y$ normalized to the smallest response so that the largest weight factor is 1. Normalization is done by multiplying the weight with the smallest response. For example the weight of a calibration point with the amount y is $(1/y) \times b$ where b is the response corresponding to the smallest amount of the calibrated compound prepared in the calibration standards. If the origin is included it is assigned the mean of the weightings of the other calibration points.
Quadratic (Amnt)	A calibration point with the amount x has the weighting $1/x^2$ normalized to the smallest amount so that the largest weight factor is 1. Normalization is done by multiplying the weight with the smallest amount. For example the weight of a calibration point with the amount x is $(1/x^2) \times a^2$ where a is the smallest amount of the calibrated compound prepared in the calibration standards.
Quadratic (Resp)	A calibration point with the response y has the weighting $1/y^2$ normalized to the smallest response so that the largest weight factor is 1. Normalization is done by multiplying the weight with the smallest response. For example the weight of a calibration point with the response y is $(1/y^2) \times b^2$ where b is the response corresponding to the smallest amount of the calibrated compound prepared in the calibration standards.
# Calibrations	A calibration point is weighted according to the number of recalibrations of the point. No normalization is done.

Quadratic calibration point weightings, for example, can be used to adjust for a spread in calibration points. It makes sure that calibration points closer to the origin, which can normally be measured more accurately, get a higher weight than calibration points further away from the origin, which may be spread.

You should base your decision, which kind of calibration point weighting to use, on your method requirements.

Calibration Table

The calibration table specifies conversions of peak areas or heights into the units you choose according to the calculation procedure you select. It contains a list of retention/migration times from a calibration run. These retention/migration times are compared with retention/migration times of peaks from a sample run. Where a match occurs, the peak in the sample is assumed to represent the same component as that in the calibration table. During an analysis or while a report is being generated, the amounts entered for each peak are used to calculate the amounts for the calculation procedure selected for the report. The type and amount of information required for creating a calibration table varies with the type of calculation procedure desired.

The following information is needed to create a calibration table:

- the retention/migration time for each calibration mixture component peak, and
- the amount of each component used in making the calibration mixture, expressed in consistent units.

Peak Summing

The peak sum table is provided for certain applications in the petrochemical and pharmaceutical industries that can be performed more effectively with the following features:

- Sum the areas of peaks that lie within a range specified by the user
- Sum the areas of a range of peaks and perform the calculations using a single multiplier
- Sum the areas of all peaks having the same name

The peak sum table is similar to, but distinct from the standard calibration table. Like the calibration table, it is associated with the current method.

| **NOTE** | You must create a calibration table for an analysis before you can create the peak sum table. |

Unknown Samples

An unknown sample is a sample containing an unknown amount of the compound to be quantified.

To find out how much of the compound is in the unknown sample, you must:

• create a calibration curve for the compound,

• inject an aliquot of your unknown sample and run the analysis in exactly the same way as for the calibration sample,

• determine from the signal the response, which is the area or height of the peak due to the unknown amount of the compound, and

• use the calibration curve to calculate the amount of the compound in the unknown sample.

For example, if the area of peak in the unknown sample is 500, you can determine that the amount in the unknown is 5 ng/µl, by using the calibration curve shown in Figure 39 on page 164.
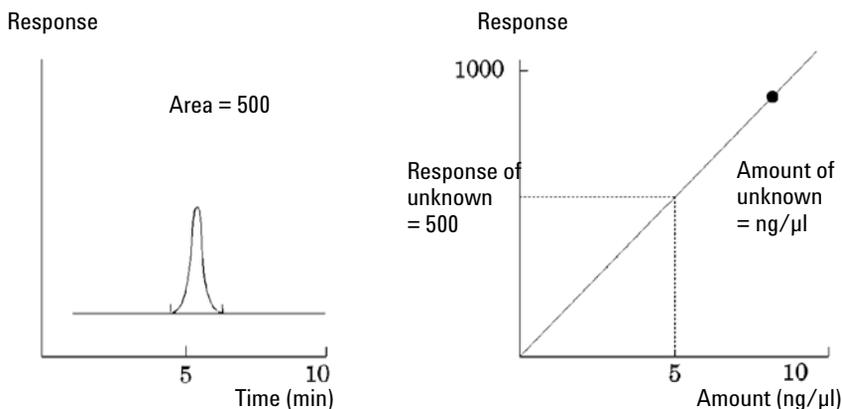
Figure 39   Signal from Unknown Sample and Calibration Curve

Recalibration

What is Recalibration?

Recalibration is the process used when you want to update a level on a calibration curve. When you recalibrate you run another sample that contains the same calibration compounds as the original, and most important, the same amount of these compounds. When you run the calibration sample, you obtain updated response factors and retention/migration times. You may also choose to average the response factors over a number of calibration runs so that response factors are weighted equally.

Why Recalibrate?

Most calibrations have a limited lifetime, due to changes in chromatography. Recalibration is necessary to maintain the accuracy of the analysis. For example, assume you have created a calibration table for the compound caffeine which you use whenever you are required to quantify samples containing caffeine. At some point you will need to replace the column/capillary. Although the column/capillary is replaced with exactly the same type, it will not behave in exactly the same way as the previous column/capillary when you first created the calibration table for caffeine. Therefore, to ensure consistency you should recalibrate the levels in the calibration table before using the new column/capillary to analyze samples containing unknown amounts of caffeine. By doing this you are quantifying samples analyzed under the same system conditions.

Manual Recalibration

You can enter peak calibration information manually and normalize the calibration table using the Manual Setup option button in the New Calibration Table dialog box. Typically, a new calibration method is produced by running a calibration standard mixture, creating a calibration table, and entering the amounts of all calibrated peaks to obtain response factors. This approach is inefficient for some application, such as found in the petrochemical industry, where the same compounds have been analyzed for many years and the response factors for various compounds and detectors are readily available.

You create the calibration table manually by entering peaks and their response factors into the calibration table, recalibrating the method using a standard that contains at least one response reference peak, and selecting Delta% update.

To reference to a specific peak for the calculation of retention time ratios, you can set this peak as a retention time ratio reference peak. All peaks with the same RT Ratio Ref # are then referenced to this peak.

Recalibrations with Peak Summing

When a recalibration is performed, the retention/migration time ranges in the Peaksum Table of the method will be updated before the actual recalibration is done. Peak Sum recalibrations are performed in this way to ensure the delta is incorporated into the time calculations.

Ways to Recalibrate

Recalibration can be done in two ways using the ChemStation software. You can recalibrate interactively or automatically during a sequence of automated analyses. Interactive recalibration is when you directly go through the process of recalibration using the ChemStation software after injecting one or more calibration samples. Recalibration using a sequence is where you specify when the recalibration occurs, but the automation software does the recalibration. For more information refer to "Automatic Recalibration" on page 107.

For information on how to perform recalibration using the software, see the How To part of the help system.

Recalibration of Unidentified Peaks

There are three ways to recalibrate unidentified peaks.

No Recalibration

If a peak in the calibration table cannot be identified in the integration results, the calibration is aborted. If this occurs in a sequence, the sequence is also aborted.

Partial Recalibration

This feature allows the recalibration of only the identified peaks. If peaks are missing, the calibration is not aborted but the report is annotated that peaks are missing.

Recalibration of all Retention/Migration Times

This feature allows the retention/migration time recalibration of all identified and unidentified peaks. This is done by using the retention/migration times of the identified peaks. There is no update of response factors for unidentified peaks.

8
Reporting

This chapter describes the concepts of Intelligent Reporting and Classic Reporting.

What Is a Report?

A report can comprise quantitative and qualitative information of the sample you analyze. The report can be a hardcopy printout, a display on the screen or an electronic file. The report can include details of the peaks detected during the run and plots of the signals that were acquired.

Reports for different purposes

You can specify reports that serve different purposes during data acquisition and data review:

- The *sequence summary report* is defined in the **Sequence Output** tab of the **Sequence Parameters** dialog. This report is automatically created by ChemStation after completing a sequence acquisition or reprocessing a sequence.

- The *single injection report* is defined in the **Specify Report** dialog. This report is created for each single sample during a sequence acquisition or sequence reprocessing.

With Intelligent Reporting, you create templates for different report types, depending on the purpose of a report. For more information, see "Report Types" on page 173.

Report destination

A report can be sent to the following destinations:

- **Screen**

  The report (including text and graphics) is displayed on the screen in the report preview window, from which it can be printed.

- **Printer**

  The report comprising text and graphics is printed on the currently selected printer.

- **File**

  The report is saved to a file, for example, an Adobe PDF file.

Classic and Intelligent Reporting

Classic and Intelligent Reporting

With Agilent OpenLAB CDS, you can choose the type of Reporting you want to use: *Classic Reporting* as it was used in the previous ChemStation versions, or *Intelligent Reporting* that provides a powerful standardized report definition language and improved reviewing functions. The following sections describe the two types of reporting.

Consequences of Activating Intelligent Reporting

If you want to use Intelligent Reporting, you need to enable Intelligent Reporting in the Instrument Configuration in the OpenLAB Control Panel.

Activating Intelligent Reporting has the following consequences for ChemStation:

- The **Report Layout** view shows the Report Template Editor for Intelligent Reporting.

- The **Review** view is visible.

- In the **Sequence Parameters** dialog, you can choose between Classic and Intelligent Reporting.

- In the **Specify Report** dialog, you can choose between Classic and Intelligent Reporting.

Intelligent Reporting

Advantages of Intelligent Reporting

Intelligent Reporting offers you the following advantages:

- You can use the **Review** view.
- Most of the functionality that is available in different settings and several dialogs for Classic Reporting is now part of the report templates. You can create or edit report templates using the **Report Layout** view, which contains the new Report Template Editor for Intelligent Reporting. The Report Template Editor offers several powerful functions:
  - You can access all result data generated by ChemStation by selecting the corresponding data field.
  - You can create your own expressions in order to make calculations with the data fields. You can use any valid Microsoft Visual Basic expression.
  - You can create expressions where you make calculations with the ChemStation Custom Fields.
  - Result Flagging: You can provide expressions to highlight specific results depending on their value.
  - Snippets: The Report Template Editor provides preconfigured report items, so-called *snippets*, that you can insert into your report template via drag and drop.
- You can use the Report Template Documentation Tool to create descriptions of your report templates.
- You can report the following values as defined by European Pharmacopeia (the peak-to-valley ratio is also available with Classic Reporting; for details on the required fields, refer to the *Reference Guide*):
  - Signal-to-noise ratio
  - Relative retention
  - Relative retention time

Report Template Editor (RTE) for Intelligent Reporting

Report Types

You can create different types of reports. Depending on the report type, different data fields are available in a report template, and the report items are grouped differently.

The following report types are available

- **Single Injection**

  The generated report shows the report items from the template separately for each injection in the current data scope. You can show the data per injection, but you cannot compare results from different injections in one table or matrix.

- **Single Sequence Summary**

  The generated report shows the report items from the template separately for each of the sequences in the current data scope. You can compare results from different injections in one table or matrix, but not the results from different sequences.

- **Cross-Sequence Summary**

  With this report type, the data is *not* automatically grouped. Therefore, you must pay more attention to the grouping of your report items, but in return you can create report items that compare data from different sequences.

Template Format

All report templates are based on the Report Definition Language (RDL), which is a standardized XML format provided by Microsoft.

To create report templates, you can either use the Report Template Editor (RTE) or Microsoft SQL Server Business Intelligence Development Studio (BI Studio):

- *RTE* provides an easy-to-use interface that helps you create report templates in a few steps. It supports all types of report items and most of the respective configuration options.

  With RTE, you cannot edit templates that have been created with BI Studio. If you need to edit such templates in RTE, please ask Agilent Customer Service.

- *BI Studio* provides the complete range of functions. However, working with BI Studio requires advanced knowledge of template development. For more information, please refer to *G4635-90007 Manual for Report Template Designers*. This manual comes with OpenLAB ECM Intelligent Reporter. Please contact Agilent to receive a copy of this manual. This manual also contains detailed descriptions of the Agilent report templates that are delivered with OpenLAB ECM Intelligent Reporter. These templates are specifically designed for usage in BI Studio and contain most of the advanced features that are not available in RTE.

  In BI Studio, you can edit any report template, no matter whether it was created with RTE or with BI Studio.

Data Fields

You can access all result data generated by ChemStation during an acquisition. For each value you can select the respective data field in which the value is stored. You can arrange the data fields in the report template according to your requirements. The available data fields are arranged in the following categories:

• Sequence

• Sample

• Injection

• Signal

• Compound

• Peak

• Calibration Curve

• Instrument

• File

• Project

Report Items

You can add various report items to a report template, depending on your requirements. For each report item you can configure several properties such as the font format, background color, expressions, etc. The following report items are available:

• Text Fields

• Data Fields

• Tables

• Matrices

• Composite Groups

• Images

• Chromatograms

• Calibration curves

• Spectra

• Charts

• Method Information

Snippets

The Report Template Editor provides snippets, multiple preconfigured report items or groups of report items, which you can insert into your report template via drag and drop.

These snippets are, for example, preconfigured tables for compound results or system suitability, chromatograms for a single signal plot or multi signal plot, or control charts for calibration accuracy or retention time stability. You can use the snippets as a starting point and adjust them according to your requirements.

Custom Calculation

In the Report Template Editor, you can either display the values of the data fields as they were generated by ChemStation, or you can calculate new values for different purposes. You can create expressions using the existing data fields and also using the custom fields.

You can store the values as variables and access these variables from a subsequent report item in the template.

The Report Template Editor provides an Expression Editor that helps you to create valid expressions. All expressions are based on Microsoft Visual Basic.

Conditional Formatting

You can configure certain properties of a field or cell, depending on the values resulting from the expression. For example, if the compound amount is shown, you can create a condition for a red background if the amount exceeds a certain value.

Demo Data

When you develop a new report template in the Report Layout view, ChemStation provides the demo data that is shown in the Report Template Editor when you edit or preview a template. The demo data corresponds to the data set (sequence or single runs) that is currently selected in the Navigation Table of the **Data Analysis** view. If you develop a template for a sequence summary report, you need to load a sequence in the Data Analysis view and select a subset of samples. If you develop a template for a single injection report, it is sufficient if you select only one sample in the Data Analysis view.

Storage of Report Templates

ChemStation provides a number of predefined report templates. These default templates are located in the directory chem32\repstyle.

For sequences, the report templates used for sequence summary reports and single injection reports are located in the result set on the same level as the sequence methods. No report templates are stored on the data file level of a sequence.

For single samples, the report template is located in the data file.

Browse Templates Dialog

If you browse for report templates in the **Sequence Parameters** dialog or in the **Specify Report** dialog, you can synchronize the templates in the Default Templates directory and in the result set.
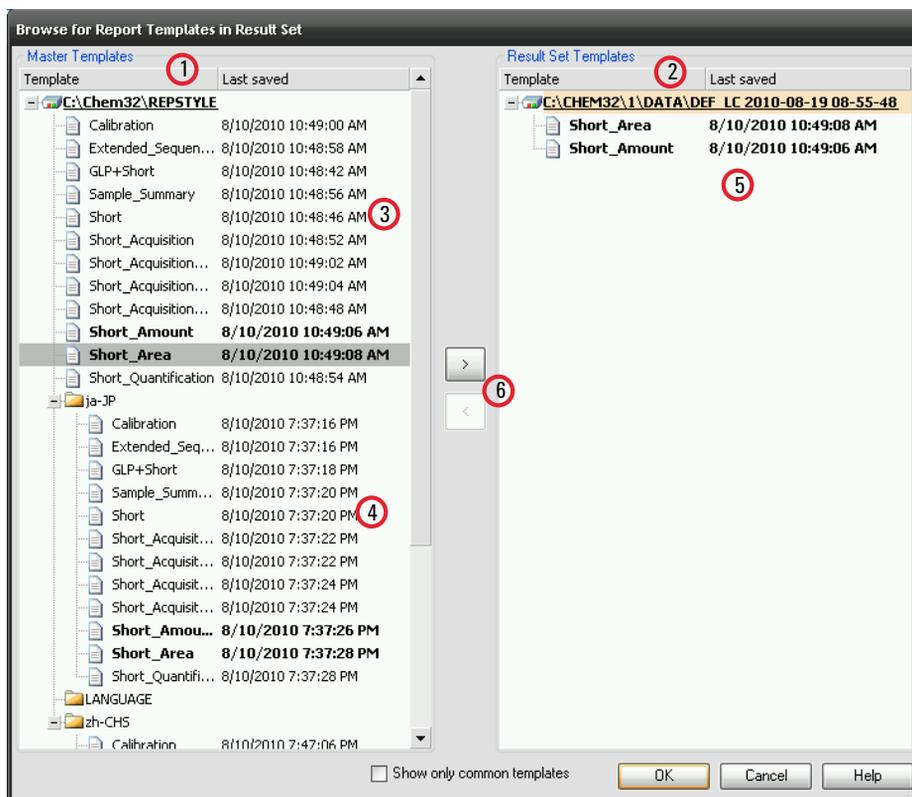
Figure 40 **Browse for Report Templates in Result Set** dialog

1   On the left side, you see the templates in the Default Templates directory (chem32/repstyle).

2   On the right side, you see the templates in the currently loaded result set.

3   For each template, you see the date when it was last saved. The tooltip of the date shows the template's last history entry.

4   Templates can also be stored in subfolders of chem32/repstyle.

5   Templates that are common for the result set and the Default Methods directory are shown in bold. The templates are matched only by name.

6   You can copy templates from the Default Templates Directory to the result set either by using Drag&Drop or by using the > button.

Data file management with Unique Folder Creation OFF

Using the Unique Folder Creation OFF option, the templates for sequence summary reports and single injection reports are always referenced from the Default Templates directory (chem32\repstyle).

Storage of Generated Reports

File Naming for Single Injection Reports

When you provide a file name for the single injection report in the **Specify Report** dialog, you can use the following tokens:

- <Date> current date
- <Time> current time
- <SeqN> sequence file name (will be "_" for a single sample)
- <Cont> name of result set (will be "_" for a single sample)
- <SamN> sample name
- <Lims> LimsID
- <InjD> injection date & time
- <File> data file name
- <SLoc> sample location

File Naming for Sequence Summary Reports

When you provide a file name for the sequence summary report in the **Sequence Output** tab of the **Sequence Parameters** dialog, you can use the following tokens:

- <Date> current date
- <Time> current time
- <SeqN> sequence file name
- <Cont> name of result set

Report Templates in Central Data Storage

If you use a central data storage system, report templates are treated as a separate document type. You can upload templates to central data storage, download templates from central data storage, or update all local report templates with the latest version from central data storage.

Classic Reporting

Reporting Results

Two types of reports are available:

- an uncalibrated report which does not correct for detector response, and

- a calibrated report that shows results corrected for the difference in the detector response to various components of the sample.

Uncalibrated Reports

The uncalibrated reports include the **Area%** and **Height%** reports. These reports are mainly used in the preparation of calibrated reports. They may be of value as a final report if the amounts of compound required to produce a unit area or height response for the compounds of interest are similar.

Calibrated Reports

Calibrated reports correct for the difference in the detector response to the reported compounds. One or more calibration samples containing known amounts of the reported compounds must be run under the same conditions used for the unknown sample. The integration data from these calibration sample(s) are used to prepare a calibration table. This is a list of retention/migration times, amounts and responses which is used in the generation of the report. The calibrated reports are based on two calibration procedures called external standard and internal standard.

External Standard Report

The ESTD report lists the results with the units of your choice or with each compound as a percentage of all the compounds present. The external standard procedure requires that the relative injected volume of both the calibration and unknown samples must be known accurately. The reliability of the external standard report is limited by the reproducibility of the injection and any other factors that might change from sample to sample.

Internal Standard Report

The limitations of the external standard procedure can be overcome by using the internal standard approach. An accurately known amount (not necessarily the same amount) of the internal standard is added to both the calibration sample(s) and unknown sample. The response of each compound of interest is divided by the response of the internal standard to give a response ratio. The calibration curves are a plot of this response ratio versus the amount ratio and this information is used in the calculation of the reported results. In this way inadvertent errors in injection volume or slight changes in the chromatographic/electropherographic system that affect all compounds similarly are cancelled out. The ISTD report lists the results with units of your choice.

Control Charts Report

The Control Charts Report tracks a single result from multiple runs for a specific calibrated compound. The **Control Chart** feature is installed after ChemStation is operational. Methods that use this feature pass the tracked result to a Microsoft Excel work sheet after each run. Excel is then used to print the report.

Quantitative Results

The report type is identified by the name of the calculation method used to prepare it, for example, an ISTD report. Each type is briefly described below. The calculations for each report are given in "Quantitative Results" on page 182.

Area% provides the simplest report and requires no calibration data since no correction is made for the difference in detector response of sample components. The Area% report is particularly useful in developing a calibration table for use with the other report options. This report is suitable for analyses in which the difference in detector response of the components is not significant.

Height% gives a report similar to the Area% report except that peak height is used for the calculations instead of peak area.

Norm% gives a report in which each component is reported as a percentage of all the components present. The peaks are corrected for detector response before calculation of the percentage of each.

ESTD produces a report of the actual amount of each substance in whatever units you choose. Amounts are calculated using a previously established calibration table. The use of an external standard requires that the injected volume of the calibration mixture be known.

ESTD% produces a report of the relative amount of each substance as a percentage of the injected sample. Amounts are calculated using a previously established calibration table. The use of an external standard requires that the injected volume of the calibration mixture be known.

ISTD produces a report of the actual amount of each substance. Amounts are calculated using a previously established calibration curve. The use of an internal standard in both the sample and the calibration mixture eliminates the need to know and control the volume of the sample injected. This also corrects for any variation in instrument performance between runs.

ISTD% produces a report of the relative amount of each substance as a percentage of the injected sample. The use of an internal standard in both the sample and the calibration mixture eliminates the need to know and control the volume of sample injected. This also corrects for any variation in instrument performance between runs.

Reporting Custom Field Values

The values of the custom fields attached to a certain sample according to its acquisition method can be added to the report. Sample custom fields are listed at the end of the report header that contains the general sample information. Compound custom fields appear at the end of the report.

Report Styles

You choose to add a signal to any of the report styles by checking the appropriate box in the Specify Report dialog box.

The following report styles are available:

- **None** - No text will be reported. The chromatogram will be reported only if the Add Chromatogram Output option is selected.

- **Short** - Comprises quantitative text results of all integrated signals which are setup in the Signal Details dialog box (LC only) or the Signal dialog box (GC only). The peak width in the short report is calculated with the more complex formula used by the integrator: PW = 0.3(IPRight - IPLeft) + 0.7(Area/Height) where IPRight and IPLeft are the inflection points.

- **Detail** - Comprises header, quantitative results, and calibration curves. The header is stored in a file called RPTHEAD.TXT in the method directory. You can change the header using a text editor to include a method specific text.

- **Header** + **Short** - Comprises file header and quantitative text results. The header is stored in a file called RPTHEAD.TXT in the method directory. You can change the header using a text editor to include a method specific text.

- **GLP** + **Short** - Comprises header, sample information, instrument conditions, logbook, signal, and quantitative results. The header is stored in a file called RPTHEAD.TXT in the method directory. You can change the header using a text editor to include a method specific text.

- **GLP** + **Detail** - Comprises header, sample information, instrument conditions, logbook, signal, quantitative results, and calibration curves. The header is stored in a file called RPTHEAD.TXT in the method directory. You can change the header using a text editor to include a method specific text.

- **Full** - Comprises header, sample information, instrument conditions, logbook, signals, and quantitative results. The header is stored in a file called RPTHEAD.TXT in the method directory. You can change the header using a text editor to include a method specific text.

- **Performance** - Produces a report according to the limits specified in the Edit Performance Limits dialog box of the System Suitability menu.

  For uncalibrated methods, the report parameters include the peak number, retention/migration time, peak area, peak height, signal description, true half-height peak-width (please refer to *True Peak Width Wx [min]* in the Reference Guide), symmetry, k', efficiency (plates) and resolution for each peak.

For calibrated methods, the report parameters include the peak number, retention/migration time, compound name, amount, signal description, true half-height peak-width, symmetry, k', efficiency (plates) and resolution for each peak.

The half-height peak calculation is not the same as the more complex peak width formula used by the integrator. The values of efficiency and resolution are based on this calculated peak width. The report header consists of all method relevant information, including instrument, column/capillary, sample, and acquisition parameters. The signal is also plotted.

- **Performance** + **Noise**- Combines the Performance report style with the noise calculations for the noise ranges defined in the Edit Noise Range dialog box of the System Suitability menu. In addition, the noise, given as six times the standard deviation, as peak-to-peak, and as ASTM noise.The drift and wander is also determined.

- **Performance** + **Extended**- Produces an extended report with all the parameters from the peak performance calculations and individual plots of each peak. The plots include the baseline, the tangents, and the peak widths at defined heights. This report type includes only calibrated peaks.

In addition to the parameters printed for the Performance report style, more Peak Performance Parameters are determined: the peak start and end peak times, skew, excess, peak width, USP tailing factor, time interval between data points, number of data points, statistical moments, plates, plates per meter, selectivity and resolution of each peak are printed. The peak width, plates, plates per meter, selectivity, and resolution are calculated by the true half height, 5 sigma, tangent and tailing methods (please refer to *Performance Test Definitions* in the Reference Guide for details).

The report header consists of all method relevant information such as instrument, column/capillary, sample and acquisition parameters and a plot of the signal. For a complete list of the peak performance parameter algorithms refer to *Performance Test Definitions* in the Reference Guide for details.

The spectral report styles (**Short** + **Spectrum**, **Detail** + **Spectrum**, **Performance** + **Library Search**) are described in *Understanding Your Spectra Module*.

Adding a Customized Report to Report Styles

You can add a custom report template that you have created in the Report Layout view of ChemStation to the list of available report styles.

NOTE	All reports apart from the performance reports list the peakwidths calculated with a more complex formula by the integrator (for details on the peakWidth calculation please refer to *Peak Width* in the Reference Guide).

Other Report Style Parameters

Summed Peaks Table

The peak sum table is provided for certain applications in the petrochemical and pharmaceutical industries that can be performed more effectively with the following features:

- Sum the areas of peaks that lie within a range specified by the user
- Sum the areas of a range of peaks and perform the calculations using a single multiplier
- Sum the areas of all peaks having the same name

When the report is generated, ChemStation uses the peak sum table to produce a peak sum report that is printed after the standard report calculations with the exception of Norm% which is replaced by the peak sum report.

Report Layout for Uncalibrated Peaks

To change the report layout for uncalibrated peaks, choose one of the following in the Specify Report dialog box.

- Separately to report the uncalibrated peaks in a separate table if sorting by retention/migration time is selected, or separate tables if sorting by signal is selected.
- With Calibrated Peaks to report the uncalibrated peaks together with the calibrated peaks.
- Do Not Report to suppress the reporting of uncalibrated peaks.

Sequence Summary Reporting

Overview

ChemStation can print a variety of standard reports for individual sample analyses. Sequence summary reporting is an additional way of reporting, that allows you to calculate and report parameters across a number of different analyses. It is useful, for example, to test the stability of an instrument or the robustness of a new method.

A sequence summary report may include:

- a title page,
- instrument configuration, including the revision numbers of the instrument and details of the analytical column/capillary being used,
- sequence table listings which describe what the automated sequence of analyses should have done,
- logbook descriptions of what the sequence actually did and any unexpected events which occurred during the sequence,
- method listings,
- individual reports on each sample,
- statistics on the analyses based on selected criteria—*statistics are calculated only for calibrated compounds*, and
- a table of contents with page number references to the detailed sections of the report.

Setting up a Sequence Summary Report

When setting up a sequence summary report, you can select any combination of the following nine categories by activating the appropriate check boxes and, where appropriate, selecting a report style from the template selections. Each template specifies the contents and layout of that particular section of the whole sequence summary report.

You can choose one of the following sequence summary report styles:

One Page Header

The GLP template prints GLP in large letters as a title page for the following report. It also includes the date and a place for a signature.

Configuration

Select **Configuration** if you want to include the instrument configuration and analytical column/capillary specifications in the report.

Sequence Table

Select **Sequence Table** to include a list of the samples, sample quantification parameters and method names in the report. This list shows what the system should have run.

Logbook

Select **Logbook** for a listing of the analyses the system ran, including instrument conditions and any unusual events that occurred while the samples were being analyzed.

Methods

Select **Methods** to list all the analytical methods used in the series of automated analyses.

Analysis Reports

Select **Analysis Reports** to get individual analysis reports according to the report style set up for the method.

Individual analytical reports may be printed after each analysis according to the report style specified for the method in question, in addition to the report sections specified in **Sequence Summary Reporting**. See "Sequence Output" below.

SUILabel Type = Application > Statistics for Calibrated and Sample Runs

Selecting the Statistics cal. runs will produce statistical trend analyses for calibration samples. Selecting the **Statistics** sample runs will produce statistical trend analyses for sample (unknown) analyses. Both selections have Standard Statistic and Extended Statistic template styles. **Extended Statistics** prints the statistical trends of the analyses as graphs, whereas the **Standard Statistics** selection prints only text. The selections that you make in the **Items and Limits for Extended Statistics** dialog box are used only when you choose the **Extended Statistic** option(s) in the **Sequence Summary Parameters** dialog box.

If you choose the **Standard Statistic** option(s) in the **Sequence Summary Parameters** dialog box, then the statistics reported are:

- retention/migration time,
- area,
- height,
- amount,
- peak width (based on the report style, see "Report Styles" on page 184)
- symmetry.

The statistic calculation does not distinguish between different calibration levels in a sequence that uses multilevel calibration methods. This means that the concentration-dependent items, for example, Area, Height, Amount (see the dialog box for Items and Limits for Extended Statistics) are all taken together, regardless of the calibration level. The **Statistics for Calibration Runs** values are therefore not useful for multilevel calibration methods in sequences.

Summary

The **Summary** selection will print an overview of the series of samples analyzed and the methods used. If the Summary choice is selected together with other Sequence Summary selections, page numbers referring to the other parts of the sequence summary report are included. Two Summary styles are available:

The **Sample Summary** tabulates details of the sample analyses run in the sequence, with some sample information such as the sample name, data file name, method and vial number.

The **Compound Summary** tabulates the sample analyses with basic quantification results for each calibrated compound, or each peak, depending on the type of report specified in the method.

Sequence Output

In the **Sequence Output** dialog box you can define where the sequence summary report should be printed.

Select **Report to file** and enter a file name to print the report to the specified file. The default setting is that data are saved to the file GLPrprt.txt. In GC systems with dual injection the data are saved in GLPrptF.txt and GLPrptB.txt for the front injector and back injector respectively.

Select **Report to PDF** to save the report as a PDF document. The report is saved in the sequence folder with the name CLPrprt.PDF

Select **Report to HTM** to print the report in HTML format. The report is saved into an HTM directory in the data directory specified in the **Sequence Parameters**. The HTM report consists of an index file (index.htm) and at least two other files, a contents file (contents.htm) and a GIF (Graphics Interchange Format) file for each page of the report (e.g. page1.gif). To view the html report, open the index file using your browser.

Select **Report to printer** to print the report on the system's printer. Print individual reports for each run also activates printing of sample reports after each analysis. These reports are printed in addition to the reports specified for the sequence summary report which are produced at the end of the entire sequence. You can specify a new destination for these reports in the **Sequence Output** dialog or use the destination specified in the individual methods.

Report File Formats

A report can be saved in different formats. Each format has a specific extension. It is possible to select more than one format for a report.

.TXT   The report text is printed as a UNICODE text file.

.EMF   Each report graphic (signal or calibration curve) is saved in a Microsoft Windows metafile (WMF). Several .WMF files for one report are possible. The generated file format adheres to the Microsoft standard metafile format as defined in the Windows software development documentation. These files are compatible with the Aldus Placeable Metafile (APM) format used by a number of proprietary software packages.

.DIF   The tabular report data is saved in Data Interchange Format (DIF). This format is accepted by spreadsheet programs, for example, Microsoft Windows EXCEL. Independent from the report style selected, only the information contained in the report style Short will be saved.

.CSV   The report is in Comma Separated Values (CSV) format. This is a very simple format for tabular data that is accepted by many spreadsheet programs and databases. Independent from the report style selected, only the information contained in the report style "Short" will be saved.

There can be several .DIF and .CSV files for a single report. For each report block, the first file, for example, REPORT00.CSV, contains the report header information. Subsequent files contain the tabular results.

If the results are sorted by retention/migration time, only one file is required for the complete table, for example, REPORT01.CSV.

If the results are sorted by signal, a separate table is required for each signal. In this case, the files are named Report01.CSV through ReportNN.CSV, where NN is the number of the signal.

.XLS   The report is exported to a Microsoft Excel spreadsheet in (XLS) format. The data generally requires additional processing.

.PDF   The report is printed to a .pdf file. The ChemStation setup installs a PDF printer, called "PDF-XChange 4.0". This Printer will only be visible in the **Start Menu**/**Settings**/**Printers and Faxes** until the computer is restarted. When ChemStation is started, another temporary printer is created called "ChemStation PDF" based on the PDF-XChange printer. While any ChemStation session is running, ChemStation PDF will be listed in the **Start Menu**/**Setting**/**Printers and Faxes**. The option **Unique pdf file name** allows to store the .pdf reports independent of the reports, with file names <sequence_container_name>_<data_file_name>.pdf

9

CE specific Concepts and Functions

This chapter is relevant only if you use ChemStation to control CE instruments.
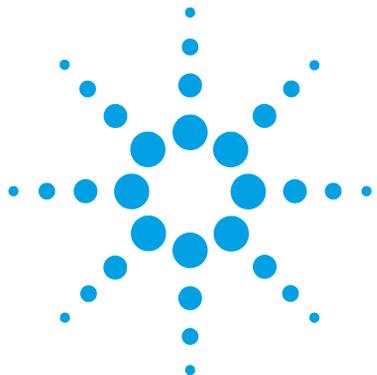
Agilent Technologies

CE Agilent ChemStation Specific Functions in Method and Run Control View

Vial Table

> **NOTE** The **Vial Table** functionality is available in the online ChemStation session only.

The **Vial Table** is a table that associates vials in the vial tray with samples, and, more importantly, with task specific vials such as buffers, flush vial, clean tubes vial, and the waste. The **Vial Table** is linked to the Sequence Table. When a sequence is loaded, the information from the Sequence Table is copied into the Vial Table. However, Vial Table entries are not transferred back into the Sequence Table. The **Vial Table Advanced Settings** dialog box is displayed when you choose the **Advanced** button in the **Vial Table**. It allows you to enable warnings of conflicts between the **Vial Table** and the method or sequence and the use of symbolic names. You must select **Enable vial table checks and warnings** to check for conflicts between the **Vial Table** and the method and sequence.

When a method or a sequence is loaded, a consistency check is made between the vial allocations in the **Vial Table** and those in the method or sequence. If there are any vial conflicts they can easily be resolved by using the **Conflict** tables.

> **NOTE** Position 49 in the vial tray is used for the needle wash vial and position 50 is left empty to allow the vial lift to return. These positions are not available in the **Vial Table**.

The **Used in** column of the Vial Table enables the usage of the vial to be specified. There are five valid entries for the **Used in** fields:

Don't Care	No consistency check is made
Method	The vial is referenced in the method
Sequence	The vial is referenced in the Sequence Table

System   This is a special vial belonging to the system configuration. The **Name** must be one of the following symbolic names:

- **@INLET** the inlet vial
- **@OUTLET** the outlet vial
- **@FLUSH** the flush vial
- **@WASTE** the waste vial
- **@clean tubes** the vial used for cleaning the replenishment tubes
- **@USER X** (where as X can be 1 to 10) the sequence placeholder

   This option allows the specification of individual vial numbers for the symbolic names used in the method. This enables the user to specify different vials for Inlet Home, Outlet Home, Replenishment, Preconditioning, Postconditioning, etc. for each line in the sequence.

Not Used   There is no vial in this position

Method Conflict Table

The **Method Conflict Table** is displayed when you load a method that has vials defined that conflict with the vials defined in the Vial Table. The **Method Conflict Table** has two sides; the left half contains an image of the **Vial Table**; the right half shows the conflicting vials.

To resolve the conflicts you can select to either replace (single arrow) or to move the vial from the method to the next free position in the **Vial Table** (double arrow). This can be done for each conflicting vial in the table.

When user-defined vials (with symbolic names @User1, @User2, etc.) are used the conflict test cannot be performed on these vials, because without the sequence information it cannot be decided whether a conflict exists or not.

Sequence Conflict Table

The **Sequence Conflict Table** is displayed when you set up or load a sequence that has vials defined that conflict with the vials defined in the Vial Table. The **Sequence Conflict Table** has two sides; the left half contains an image of the **Vial Table**; the right half shows the conflicting vials.

To resolve the conflicts you can select to overwrite the **Vial Table** information with the information from the **Sequence Table**, or if the conflict is caused by a system entry it cannot be overwritten. You can select to close the **Sequence Conflict Table** without resolving the conflicts.

When user-defined vials (in the columns User1, User2, etc.) are used the conflict test cannot be performed on these vials, because without the method information it cannot be decided whether a conflict exists or not.

Method Simulation

You can use the simulation function to check your method. During simulation the diagram reflects the actions that would be performed during the method, i.e. the vials specified in the method are shown in the lifts, power and voltage applied is shown as it would during an actual run. Simulation is faster than running an analysis - each step takes about 3 seconds. A step is defined by a change in the CE diagram.

To start simulation, load the method you want to simulate and select **Simulation** from the **Instrument** menu.

Peak Top Type

Unlike LC, GC, or MS peaks, it is quite normal for CE peaks to be asymmetrical. Because of this, is very important to have the ability to select integration parameters that will yield the highest level of accuracy and reproducibility in your quantitation results.

The following peak top types are available when selecting **Peak Top Type** in the **Integration** drop-down menu:

Highest Point

- select when the peak is triangular, and
- when you work with different concentrations

Parabolic Interpolation

- use for tailing, unseparated peaks

Center of Gravity

- provides more accurate calculations with triangular shaped peaks
- samples with similar concentrations

Gauss Fit

- use for symmetric peaks

Calibration Types

Standard Calibration is based on peak area or peak height. When you select **Standard Calibration** you have the option to **Calculate Signals Separately** or **Calculate with Corrected Areas**.

Calculate Signals Separately is selected when you want to ensure that, in the calculation of Norm% reports, the amount percent of separately reported signals add up to 100% for each signal. When **Calculate signals separately** is deselected, the amount percent of all signals add up to 100%. Selecting **Calculate signals separately** is a prerequisite for sorting by signal in the calibration table.

Select **Calculate with Corrected Areas** to make a correction to the peak area based on the migration time. In this mode, the area is divided by the migration time which can improve reproducibility in quantitative analysis when migration times are unstable.

In addition to the Standard Calibration, there are 3 capillary electrophoresis specific calibrations that are migration time based one signal.

The following calibrations types are available in the drop-down list for your Calibration Table:

- **Standard Calibration**
- **Protein Molecular Weight Calibration**
- **DNA Base-Pair Calibration**
- **Capillary Isoelectric Focusing Calibration**

For more information on the capillary electrophoresis specific calibrations, refer to *OpenLAB CDS ChemStation Edition — Reference to Operation Principles.*

Migration Time Based Calibrations

Using Migration Time Based Calibrations in a Sequence

Migration time-based calibrations and recalibrations can be included in a sequence, but only explicit calibrations and cyclic recalibrations are supported; bracketed recalibration is not supported. There is no Sequence Summary report with migration time-based calibrations.

Report Styles for Migration Time Based Calibrations

The report styles available for migration time based calibrations are limited to **Short** (quantitative text results) and **Full** (header, sample information, instrument conditions, logbook, quantitative results and peak purity plot).

Calibration using Mobility Correction

Slight changes in buffer composition, run temperature or viscosity, as well as adsorption to the capillary wall, can influence the Electro Osmotic Flow (EOF) and cause it to be unstable. The resulting change in the EOF can create a rather high standard deviation of migration times. Corrections for mobility can significantly reduce the effect of run-to-run migration time shifts by monitoring the migration time of a mobility reference peak and in turn significantly increasing the migration time reproducibility.

The mobility reference peak should be chosen with the following priorities:

*   Select peak with the highest signal
*   Select the most isolated peak
*   The EOF marker or internal standard can also be used as the mobility reference peak
*   Enlarge the search window to always find the mobility reference peak
*   If several peaks fall in the search window, the peak with the highest signal is automatically chosen as the mobility reference peak.

There are two mobility correction types available:

Effective Mobility Correction

Effective Mobility Correction uses the effective mobilities of all peaks and requires the availability of the voltage ramp data together with the electropherogram. In addition, working with effective mobility correction allows the true effective mobilities for all sample components to be determined.

Relative Mobility Correction

Relative Mobility Correction can operate in the absence of voltage data and would then assume a constant voltage for all measurements.

CE-MSD

Background Subtraction

When you select the **Subtract Background** (BSB) menu item, the most recently selected mass spectrum is subtracted from each point in the current electropherogram. The resulting data is saved in the same directory and with the same name as the original data file; however, the file extension is changed to .BSB.

The new data file becomes the current data file and the background subtracted electropherogram is displayed. A record of the number of background subtractions that have been performed is kept in the Operator item of the data file header.

If you view a tabular listing of BSB data, you may observe differences due to the precision of data representation.

| NOTE | The HELP text files in the LC/MSD refer only to LC parameters and not CE. Some features that are available in the LC/MSD software are either not available or not applicable to CE/MSD applications but are used in LC. The function **peak matching** is not applicable for CE-MS and is therefore not active. In CE-MS, UV and MS detection occurs at different effective lengths of the separation capillary. Because of the different resolution at different effective lengths, peak matching is not possible. |

Method Subdirectories for different CE Modes

Methods in CE are dependent on CE mode selected. Therefore they are stored in different subdirectories in the Method subdirectory:

CE   Stores methods for CE mode

CEC   Stores methods for the CEC mode

CEp   Stores methods for the CE plus pressure mode

CEMS   Stores methods for the CE MS mode.

CEMSp Stores methods for the CE MS plus pressure mode.

Index

In This Book

This guide describes various concepts of Agilent OpenLAB CDS ChemStation Edition. It is intended to increase your understanding of how ChemStation works. It contains information on the following subjects:

- Basic Concepts
- Data Acquisition
- Automation/Sequences
- Run Queue and Queue Planner
- Data Analysis and Review Concepts
- Calibration
- Reporting
- CE Specific Concepts and Functions

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