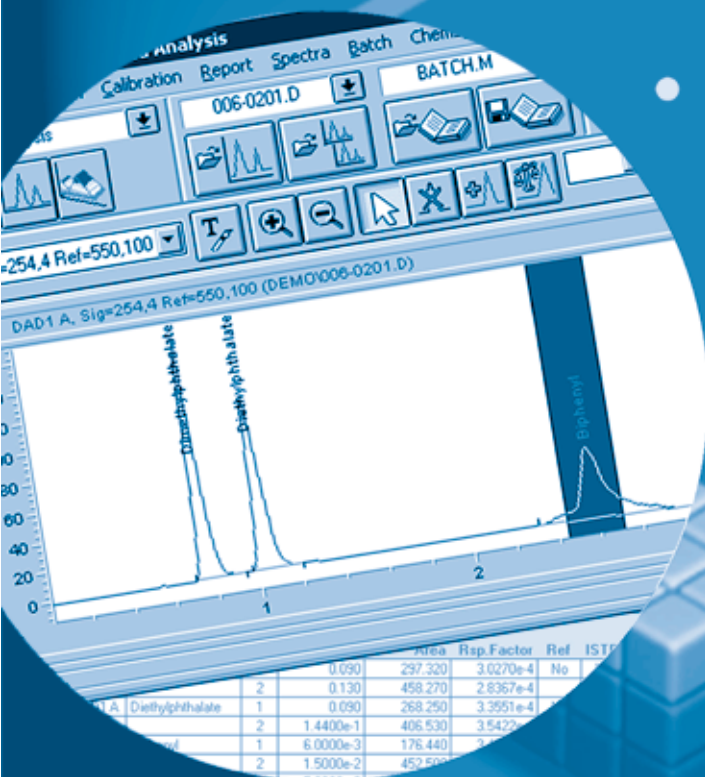


Agilent ChemStation

Understanding Your ChemStation



Agilent Technologies

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This handbook is for
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Understanding Your ChemStation

In This Book

This handbook describes various concepts of the Agilent ChemStation. It is intended to increase your understanding of how the ChemStation works.

For information on using the ChemStation please refer to the help system, especially the How To section, and the integrated tutorial.

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**Agilent ChemStation
Features**

Agilent ChemStation Features

This chapter describes the main components and features of the ChemStation. For task-specific information about your ChemStation, see the online help or the ChemStation tutorial supplied with the software.

General Description

The ChemStations for GC, LC, LC/MSD, CE and A/D systems are instrument control, data acquisition and data evaluation systems for

- HP 5890 Series II and Agilent 6890 Series gas chromatographs,
- Agilent 1100 Series modules and systems for LC,
- Agilent 1100 Series LC/MSD,
- HP 1090 Series liquid chromatographs,
- HP 1050 Series liquid chromatography modules,
- Agilent capillary electrophoresis systems, and
- Agilent 35900C/D/E dual channel analog-to-digital interfaces.

The software is designed to run on IBM-compatible personal computers under Microsoft® Windows NT 4.0 and Microsoft® Windows 2000 operating environment.

The software is sold as single instrument basic ChemStation in five versions. All versions include data acquisition, instrument control, data analysis (integration, quantification and reporting), automation and customization for one analytical instrument. An instrument is defined as running on a single timebase, but can collect data from a number of different detectors simultaneously. The five versions are:

- a single instrument ChemStation for gas chromatography (GC) systems, product number G2070AA,
- a single instrument ChemStation for liquid chromatography (LC) systems, product number G2170AA,
- a single instrument ChemStation for liquid chromatography/mass selective detector (LC/MSD) systems, product number G2710AA,
- a single instrument ChemStation system for capillary electrophoresis (CE) systems, product number G1601A, and
- a single instrument analog-to-digital (A/D) ChemStation for analog data acquisition with external event control, product number G2072AA.

General Description

The instrument control capability of the ChemStation software may be expanded by purchasing additional instrument data acquisition and control modules to allow multiple instrument, mixed technique configurations.

Additional Instrument Modules

The additional instrument modules are:

- additional GC instrument control and data acquisition module, product number G2071AA,
- additional LC instrument control and data acquisition module, product number G2171AA,
- LC/MSD instrument control, data acquisition, and data evaluation add-on module, product number G2715AA,
- additional CE instrument control, data acquisition and data processing module, product number G2172AA, and
- additional analog data acquisition module, product number G2073AA.

Additional Data Evaluation Modules

The data processing capability of the ChemStations may also be expanded through the purchase of additional data processing modules for specialist applications:

- additional diode array detector (DAD) spectral evaluation module, product number G2180AA,
- additional ChemStore sample organization and results database module, product number G2181AA, and
- LC/MSD deconvolution and bioanalysis data evaluation module, product number G2720AA, for use with the LC/MSD ChemStation only.

Up to four chromatography instruments may be configured on each ChemStation. If instruments with spectroscopy detectors (diode array detectors for liquid chromatography or capillary electrophoresis) are configured, no more than two diode array detectors are supported on one ChemStation and the number of supported instruments is restricted to three. When the ChemStation for LC/MSD is used to control the Agilent 1100 Series LC/MSD module (optionally with one Agilent 1100 Series LC or HP 1090 Series II LC), no other instruments are supported on the PC.

Data Evaluation-only Products

There are also three data evaluation-only products available that may not have instruments configured. They are designed to be used for data evaluation in an office environment:

- the ChemStation for data evaluation, product number G2090AA, has the same data evaluation capabilities as the basic ChemStations,
- the ChemStation for LC 3D data evaluation, product number G2190AA, includes diode-array spectral data evaluation and the capabilities of the ChemStation for data evaluation, and
- The ChemStation for LC/MSD data evaluation, product number G2730AA, includes diode-array spectral data evaluation, mass spectral data evaluation, as well as the capabilities of the basic ChemStation for data evaluation.

ChemStation Hardware

For details of ChemStation hardware, see *Installing Your ChemStation*.

About the ChemStation Software

Operating System

The ChemStation requires either Microsoft Windows NT 4.0, updated with Microsoft Service Pack 6a (SP6a), or Windows 2000 SP2 operating system.

The ChemStation Control Charts feature requires MicroSoft Excel 97.

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 Chapter 10 “Automation” and the online help system.

System Configuration

The configuration of the instrument system is done through 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 color definition for the ChemStation software. For further information, see the handbooks supplied with the additional ChemStation modules.

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).

The ChemStation provides commands and functions to construct, expand, extract and, where it does not alter primary data, edit registers. For further information, see the *Macro Programming Guide* which is available as online help.

Software User Interface

The ChemStation user interface is designed into Views that group software functionality according to typical analytical tasks. The following three 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 reviewing and reevaluating data that has been acquired,
- the Report Designer view for designing specific report layouts, and

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 can be installed when it is desirable to have instrument operators run samples from an easy to use, preconfigured table.

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, that may be configured for single runs or automated runs, and a schematic instrument interface diagram for GC, LC and CE 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 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 Report Designer view allows the user to graphically define the layout of a specific report style in a graphical object orientated fashion. It also uses a set of toolbars specific to this task.

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.

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 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.

The complete functionality of the ChemStation can be used during an analysis through the off-line copy.

A snapshot command is available for users who wish to start processing data before the analysis is completed.

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 Chapter 3 "Data Acquisition" and the online help system.

Data Analysis — Display

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

About the ChemStation Software

- single or multisignal displays selectable when loading the chromatogram,
- overlays of chromatograms from different samples,
- subtraction of one chromatogram from another,
- graphical vertical and horizontal alignment of signals to help visual comparison,
- signal inversion or mirroring to help visual comparison,
- graphical zoom and scrolling functions,
- adjustment of display attributes including selection of tick marks, baselines, axes, retention times and compound names (the user 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 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 includes two integration algorithms. The traditional integration algorithm was included in earlier versions of the ChemStation and is also included in most other Agilent Technologies analytical data evaluation software. The enhanced integration algorithm is the first revision of a new generation aimed at improved ruggedness, reliability and ease-of-use. In this software revision we recommend using the traditional algorithm for existing validated methods and the enhanced algorithm for new methods.

Common Integration Capabilities

Both integration algorithms include the following *key capabilities*:

- an autointegrate capability used to set up initial integration parameters,
- the ability to define individual integration event tables for each chromatographic signal if multiple signals or more than one detector is used,
- interactive definition of integration events that allows users to graphically select event times,
- graphical manual or rubber-band integration of chromatograms or electropherograms requiring human interpretation (these events may also be recorded in the method and used as part of the automated operation),
- display and printing of integration results, and
- the ability to integrate at least 1000 peaks per chromatogram.

Both integration algorithms include the following *groups of commands*:

- integrator parameter definitions to set or modify the basic integrator settings for area rejection, peak width and identification threshold (a noise rejection parameter),
- baseline control parameters, such as force baseline, hold baseline, baseline at all valleys, baseline at the next valley, fit baseline backwards from the end of the current peak,
- area summation control,
- negative peak recognition,
- tangent skim processing including solvent peak definition commands, and
- integrator control commands defining retention time ranges for the integrator operation.

Enhanced Integration Algorithm

The enhanced integration algorithm has improvements for:

- peak baseline allocation on wandering baseline chromatograms or electropherograms,
- detection of “negative areas” in tangent-skimmed peaks (tangent peaks), with construction of a modified baseline to eliminate the problem,

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- optional measurement and reporting of areas between baseline and signal that are not assigned to any recognized peak,
- additional initial parameters to remove noise generated peaks through the initial peak height parameter,
- better peak allocation on noisy signals,
- peak shoulder allocation through the use of second derivative or degree of curvature calculations, and
- ease-of-use — the new integrator algorithm has a new user interface based on tool bars and automatically focusing on key information.

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 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 further information on calibration and quantitation, see and Chapter 9 "Calibration".

Data Analysis — Standard Reporting

A standard set of user-definable report styles for sample reporting can be selected from the report specification screen. Every standard report type contains standard information groups and optional information groups.

For more information on the report styles available, see Chapter 12 "Using the ChemStation Reports".

Data Analysis — Specialized Reporting

Advanced reporting capabilities are also included in the ChemStation for applications that require a more specialized set of reports. These include statistics on separation quality, reports that include trend analysis between samples and user-defined report layouts.

System Suitability Reports

System suitability reports enable users to report system performance parameters for individual analyses. There are three variations, or styles of these reports.

The **standard Performance report** prints parameters for uncalibrated methods that include:

- retention time,
- capacity factor (k'),
- peak area,
- peak height,
- symmetry,
- true peak width at half height,
- efficiency in plate numbers,
- resolution, and
- selectivity.

For calibrated methods the compound name and amount replace the peak area, height and selectivity columns.

The report header includes the standard header and footer, sample information block, the analytical column parameters and optionally a plot of the chromatogram.

The **Performance and Noise style** adds an evaluation of the signal noise, in up to seven user-defined evaluation ranges, to the data from the Performance report style. The noise parameters are reported as signal to noise ratios for each peak or calibrated compound and a noise table for each signal. Each noise table includes noise calculated by the six times standard deviation, peak to peak and ASTM methods as well as the wander and drift.

The **Extended Performance** style adds plots of each individual peak showing graphically the peak start and stop times, half width and baseline.

This style includes the following parameters in addition to the ones reported by the standard Performance reports:

- area, height and amount,
- skew,
- excess,
- usp tailing factor,
- time interval between data points and number of data points over the peak,
- statistical moments (M0 to M4),
- peak width at half height calculated by the true, five sigma, tangent and tailing methods, and
- plate/column and plates/meter calculated by the peak width at half height, five sigma, tangent and statistical methods.

Users may define their own noise evaluation ranges and acceptable limits for these performance criteria. Values lying outside the user-defined acceptable limits are indicated on the report.

For more information on system suitability calculations, see Chapter 13 “Evaluating System Suitability”.

Sequence Summary Reports

Sequence summary reports are produced at the end of a series of automated analyses. Their range of application is from a brief summary of the samples analyzed to a detailed graphical repeatability or trend analysis of user-selectable parameters between different samples, analyzed by the same method. The reports are built up from nine optional categories of information:

- a header page that may be user defined,
- the instrument configuration including revision numbers and analytical column or capillary specifications for LC and CE systems,
- the list of samples scheduled for analysis; the sequence,
- The logbook printout which states what was analyzed and documents the data acquisition and processing steps as well as any unexpected events,
- a printout of the analytical methods,
- individual sample reports,

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- statistics on calibration samples, and
- statistics on unknown samples.
- a summary page that may be either a sample summary, one line of information per analysis, or a compound summary with a short compound summary table in addition to the sample summary.

For more information on sequence summary reporting, see the online help system and “Sequence Summary Reporting” on page 237.

Customized Reports

A customized reporting design view is included in the ChemStation for users who want to define the exact content of their own reports. The user graphically defines a report layout which may include general sample information, signal, integration and quantitative analytical result information. The user may define individual elements, such as text, tables and graphics, organize them in information sections and graphically adjust their relative position size and orientation of each defined element. The individual sections may be added, deleted, reordered and nested.

The user may define headers and footers to appear on every page, time stamps for the report and page numbering in the *page x of y* format. The information included in the report may be any ChemStation or user-defined parameter.

Once the report has been designed it may be associated with a particular method to make it the default report format for that particular type of analysis.

Customized reports may be output to the screen, printer and to a file. Reports to the screen include graphics.

For more information on the report layout, see the online help system.

Control Chart Reports

A Control Chart feature is included with the ChemStation software. Once this feature is installed and selected, the user may automatically track a selected parameter of a compound each time a method is run. These parameters include: Amount, Response Factor, Retention Time, and Area.

For more information on these Custom and Control Chart reports, see the online help system.

Utilities and Compatibilities

General

The 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).

The 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.

The ChemStation includes commands and functions to support the open database connectivity (ODBC) standard defined by Microsoft. The ODBC standard is supported at extension level one. Further information may be found in the specifications of the ChemStore product.

ChemStations for LC and CE

Methods, data files, spectral libraries and sequences from the previous versions of the ChemStations for LC, revisions A.01.00 to A.04.02 inclusive, are compatible with the ChemStation software.

The ChemStation software includes file conversion utilities for LC ChemStation (Pascal Series) liquid chromatography data files and spectral libraries. They can be run automatically from macros (see the customization section) to convert chromatographic data into the new ChemStation for LC format. Note, however, that the conversion utilities are only supported by Windows 95.

Transferring data between the Pascal and DOS environments can be done by using 3.5-inch disks, serial point-to-point connections or a local area network.

HP 3365 ChemStations

Methods, data files and sequences from the HP 3365 ChemStation (DOS Series) family must be converted using the appropriate import function built into the ChemStation. After the conversion process both the methods and data files are compatible with the ChemStation. Imported sequences can be used for reprocessing data files only.

Customization

The ChemStation can be customized using the 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 *Macro Programming Guide* which is available as online help.

The ChemStation supports Microsoft's ODBC protocol with commands to establish and close database links, return the status of a particular link, add rows to a database table and execute structured query language (SQL) statements.

Automation

The ChemStation can execute multimethod 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 seven 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, analysis methods, sample quantification parameters including sample amount, a multiplier and dilution factor, calibration specification and the number of repeat injections. 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 unknowns, calibration 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, and
- 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 Chapter 10 "Automation" and the online help system.

Good Laboratory Practice

The 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. It is documented together with onsite revalidation protocols in the *Validation Binder Agilent ChemStation for LC*.

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 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.

The ChemStation may be configured for restricted access for two user access levels, an operator and manager level. The manager level may be password protected and allows access to the complete ChemStation functionality. The operator level restricts the user to key functionality and executing defined analytical methods. The operator level is intended for use in routine laboratories and specifically prevents users from modifying and creating new methods.

Method Robustness

- Sequence summary reports (see “Data Analysis — Specialized Reporting” on page 23) provide a means to test methods for robustness. The extended format reports for user selected criteria, are reported as trend charts and may be used to determine the realistic operation limits. 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

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 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 allows the complete reconstruction of the reported data at a later date. The method is saved at the completion of all the analytical steps.

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 (see the Reporting section above).

GLP Save register files, specified as part of the method configuration, save all the original data, including sample information, data analysis method, chromatographic 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.

Instrument Control

The instrument control capability of the ChemStations may be expanded through the purchase of additional instrument modules to allow multiple instrument, mixed technique configurations. For further information, see the handbook(s) supplied with the additional ChemStation modules.

Networking

The ChemStation is tested and supported with Agilent's LanManager software, Novell NetWare, Microsoft Windows NT and Microsoft Windows 2000 products based on the IEEE 802.3 CSMA/CD specification. It should be compatible with any networking software compatible with the programming standards of Microsoft Windows.

These products enable the ChemStation to share physical devices such as plotters and printers with other laboratory computers as well as sharing information such as data files and methods.

Client/Server

The ChemStation software may be installed on a suitable network server and downloaded onto the client PCs as required. Each client specific configuration ensures a suitable environment for different techniques and individual users while the centralized software installation relieves the burden of managing many copies of the same ChemStation installation in one work environment.

LAN-based instrument control

The ChemStation software provides LAN-based instrument control and data acquisition for the Agilent 6890 GC, the Agilent 35900E A/D control module and the Agilent 1100 Series LC. You can easily control and monitor instruments by connecting them to a LAN on which the ChemStation PC resides. This arrangement allows the ChemStation PC to be located remotely from the instruments it controls.

Documentation

The documentation set contains specific sections on:

- Installing and learning the ChemStation software,
- Using the ChemStation software,
- Understanding the principles of how the software works, and
- Customizing the ChemStation.

Installing and Learning

Each ChemStation software product comes with an installation manual that includes details of the key steps in PC hardware and software requirements, instrument interface installation, ChemStation installation and installation qualification. The installation manual is specific to the purchased configuration and may include troubleshooting, system records and system maintenance advice.

Each ChemStation includes a task-based tutorial that is built into the software. This tutorial is the primary learning aid and is designed to let users learn what they want at their own pace. Each analytical task is divided into a number of clear, guided steps, each of which the users may see executed automatically by the software and then practice themselves.

Using the Software

Two additional categories of online information are designed for the routine user.

The ChemStation includes comprehensive, Windows-style, context-sensitive and indexed online help. This system gives detailed explanations of every screen and the meaning of the parameters on that screen. The detailed explanations are backed up by graphics where appropriate, and may be copied to the Windows clipboard for incorporation into the users' own documentation, or for printing.

The How To part of the online help also includes check lists of the more complex technique-specific and common chromatography tasks to help less frequent users who want to be sure they set up the system correctly. These checklists are linked directly to the detailed online help information.

Understanding the Principles

The *Understanding Your ChemStation* manual documents the principles of the software operation and the algorithms used in the data manipulations.

Customization

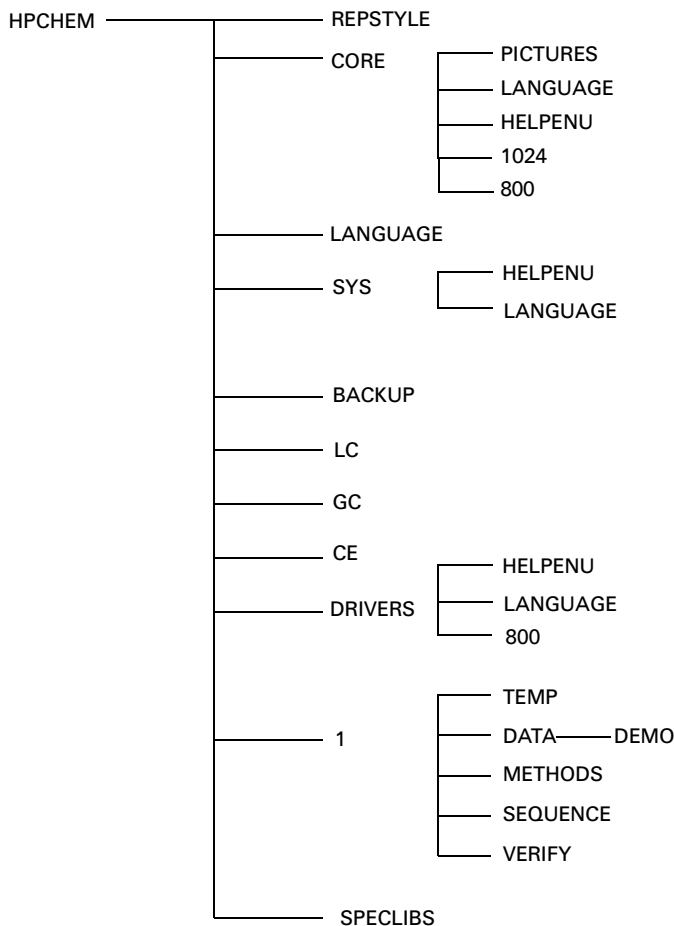
Sophisticated users who wish to customize the operation of the ChemStation, or who want to build in additional features, may do so by writing macros.

The primary reference manual, *Macro Programming Guide* which is available as online help, has a comprehensive set of functional examples backed up by a complete description of the internal data types and structures.

The commands help file, accessed directly from the ChemStation's Help menu or the Show Command dialog box, is the programmer's function reference. It includes syntax and parameter explanations with example macros illustrating the use of many of the commands. By virtue of being online, the users can copy the examples and command syntax directly into their own macro source files.

The ChemStation Directory Structure

The following example shows the directory structure of the ChemStation. It comprises generic directories that are shared by all configured instruments and instrument-specific directories. The software installation program creates a subdirectory of the ChemStation directory (by default HPCHEM) for each configured instrument with the instrument number. Inside this subdirectory, the data, methods and sequences for this instrument are stored by default.



The ChemStation Directory Structure

The ChemStation subdirectories are as follows:

Directory	Contents
HPCHEM	The directory comprises the programs to configure and start the ChemStation software. It must be part of the PATH variable. This directory is added automatically by the installation program unless you provide an alternative.
REPSTYLE	Used for report templates defined using the report designer.
CORE	Used for the core components of the software shared by all chromatographic instrument configurations. This is the working directory of the ChemStation.
PICTURES	Comprises graphics needed by the ChemStation.
LANGUAGE	Used for the language-specific code of this part of the software.
1024 and 800	Comprise initialization files for the graphical user interface. Do not change.
SYS	Comprises generic components shared by all chromatographic instrument configurations. \hpchem\sys must be part of your PATH variable. By default the installation programs adds it for you.
HELPENU	Used for the US-English version of the help files for the appropriate software part.
LANGUAGE	Used for other language specific software parts.
BACKUP	Used for backup copies of old files during installation.
DRIVERS	Comprises instrument drivers for the configured instruments.
1	Used for the configured instrument (1 to 4). This subdirectory comprises five additional subdirectories: DATA, METHODS, SEQUENCE, VERIFY, and TEMP.
DATA	Comprises all result directories of your analyses. It can also comprise more subdirectories if you structure the disk with subdirectories as you work, by defining them using the Sample Information or Sequence Parameters dialog box. Result directories are identified by a name with a .D extension. For more information on the structure of data files, see "Data Files" on page 56.

The ChemStation Directory Structure

METHODS	Comprises all method directories that have a .M extension. For further details on the contents, see “Method Directory Structure” on page 46.
SEQUENCE	Comprises the sequence tables. The sequence files in these directories have a .S extension.
VERIFY	Comprises data files, methods, and the results of data processing stored in register (.REG) files. These files execute the ChemStation verification procedure described in the online help. One set of data, method, and register files is used for each verification test
TEMP	The TEMP subdirectory comprises temporary work files and the logbook files. For example, for Instrument 1 the online logbook is called INSTR1.LOG and the offline logbook is called INSTR1-2.LOG.
LC, GC, CE	Instrument driver-specific code such as INI files. These directories are only present, if an instrument of this type is installed.
SPECLIBS	Comprises spectral libraries. (ChemStations for 3D LC systems, LC/MS systems and CE systems only).

Methods

Methods

This chapter describes the following:

- what is a method?
- parts of a method,
- status of methods,
- creating methods,
- editing methods,
- method directory structure, and
- what happens when a method is run?

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.

Parts of a Method

A method is identified by a name of up to eight alphanumeric characters. The file name always has the .M extension to identify it as a method. Methods are stored as MS-DOS 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 or electropherogram. These timed events can be used to change the way integration is done.

Peak Identification

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

Report

Defines the format of the report that is printed 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.

Status of Methods

A method can exist in two states.

Stored Method

This is a method stored on the computer disk. Stored methods have a name with up to eight alphanumeric characters followed by the extension .M.

Current Method

When a stored method is recalled from the disk it becomes the current method. There is always a current method in memory. When the ChemStation is started for the first time, the default method supplied by Agilent Technologies is always loaded as part of the startup process. For example, this may be one either:

- DEF_LC.M for an LC instrument,
- DEF_GC.M for a GC instrument, or
- DEF_CE.M for a CE instrument.

A copy of the default method is placed in memory and becomes the current method. You can load a different method at this point and it will become the current method. The default methods listed above use the enhanced integrator algorithm. If you want to use the standard integrator algorithm, you need to build your method using:

- DEFOLDLC.M for an LC instrument,
- DEFOLDGC.M for a GC instrument, or
- DEFOLDCE.M for a CE instrument.

Creating Methods

Creating a new method always means modifying the current method and saving the modifications under a new method name. Be aware that when the current method is changed, the disk version remains unchanged until you save your changes.

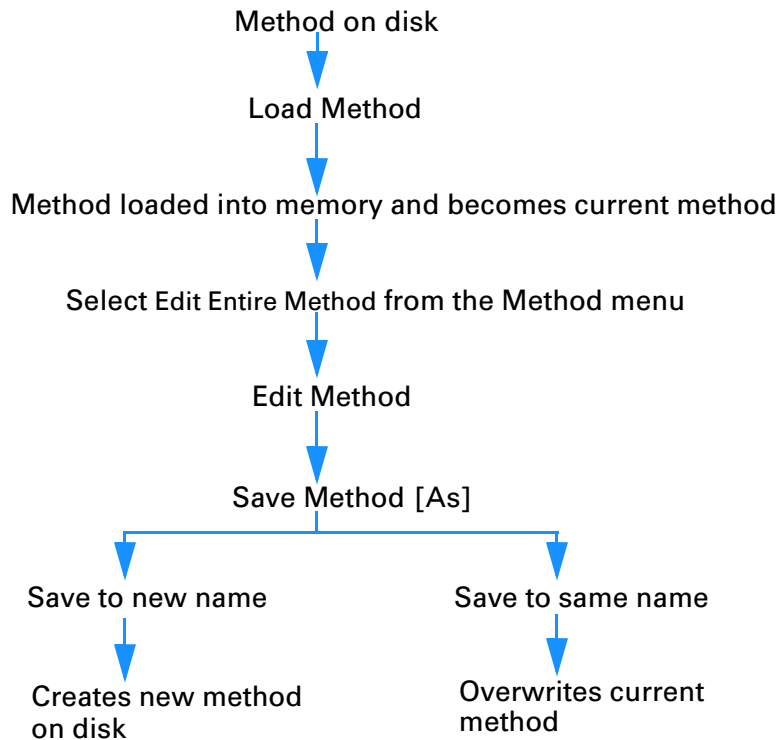
You have a choice of how to create a method. You can create a method to do either one or all parts of an analysis. For example, you can create a method to do only data acquisition. When you are ready to analyze the data and generate a library search report, you can then modify the method to do these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

CAUTION

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 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, and
 - reporting parameters.
- *Run Time Checklist* comprises:
 - the parts of the method that will be executed.

Method Directory Structure

A method comprises a group of files stored in the method directory.

The METHODS subdirectory comprises all the method subdirectories that have a .M extension.

Method files with the .MTH extension contain parameter sets and are in ASCII 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:

LC1090.MTH	Comprises the HP 1090 acquisition method.
GC5890.MTH	Comprises the HP 5890 acquisition method.
HPCE1.MTH	Comprises the capillary electrophoresis acquisition method.
DAD1.MTH	Comprises the acquisition method for the HP 1090, HP 1050 or HP 1040 diode array detectors.
FLD1.MTH	Comprises the acquisition method for the HP 1046 fluorescence detector.
ECD1.MTH	Comprises the acquisition method for the Agilent 1049 electrochemical detector.
ADC1.MTH	Comprises the Agilent 35900 acquisition method. If two identical instruments are configured, the method files are called ADC1.MTH, ADC2.MTH.
1050VWD	Comprises the acquisition method for the HP 1050 module specified in the last three characters, in this example, the variable wavelength detector.
DAMETHOD.REG	For data evaluation.
LALS1.REG	Comprises parameters for the Agilent 1100 Series autosampler. The method files for the other Agilent 1100 Series modules follow the same convention lxxx1.reg where xxx is the module acronym.

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.

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

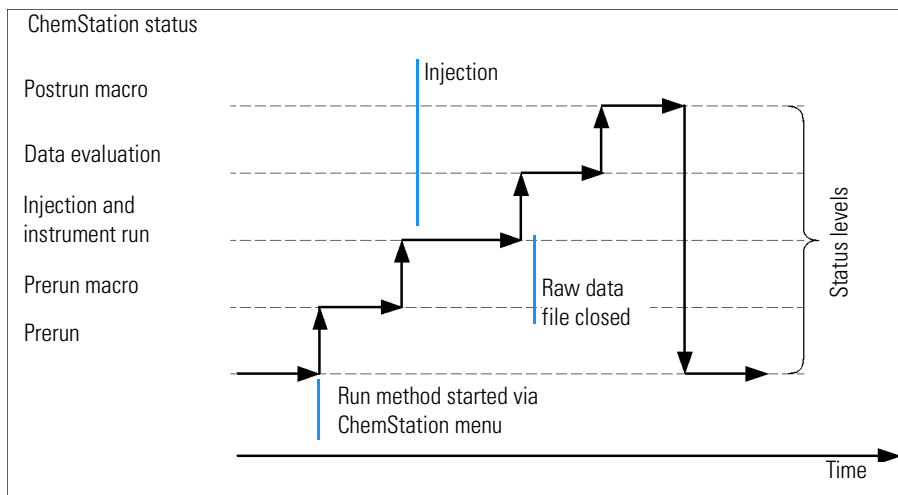
Method Operation

Figure 1 shows an overview of the ChemStation status during the method operation, where *all* parts of the Run Time Checklist are selected.

What Happens When a Method is Run?

Figure 1

Method Operation



Pre-run Command or Macro

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

- 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 or electrophoretic information, and spectral data if available.
- Data are acquired and stored in a data file.

Data Analysis

When the stop-time has elapsed, the analysis is finished and all rawdata is stored on the computer's hard disk. The data analysis part of the software starts when all the rawdata 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 (ChemStations for LC 3D Systems, LC/MS Systems and CE Systems Only)

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 (ChemStations for LC 3D Systems, LC/MS Systems and CE 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.

What Happens When a Method is Run?

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 checkbox in the runtime checklist. You can review, but not edit, GLP data in the data analysis menu of the 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 Data Acquisition is activated in the Run Time Checklist. It copies the *current* method to the data directory.

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*

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.

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.

Methods

Method Operation Summary

Data Acquisition

Data Acquisition

This chapter describes the following:

- what is data acquisition?
- data files,
- directory structure,
- online monitors,
- logbook,
- system status, and
- the system diagram.

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 the ChemStation electronically and stored in the signal data file.

Data Files

A data file comprises a group of files stored in the DATA directory as a subdirectory with a data file name and a .D extension. Each file in the directory follows a naming convention.

Name	Description
*.CH	Chromatographic/electropherographic signal data files. The file name comprises the module or detector type, module number and signal or channel identification. For example, ADC1A.CH, where ADC is the module type, 1 is the module number and A is the signal identifier and .CH is the chromatographic extension.
*.UV	UV spectral data files. The file name comprises the detector type and device number (only with diode array and fluorescence detector).
REPORT.TXT	Report data files for the equivalent signal data files. The file name comprises the detector type, device number and signal or channel identification, for example, ADC1A.TXT.
SAMPLE.MAC	Sample information macro.
RUN.LOG	Logbook entries which have been generated during a run. The logbook keeps a record of the analysis. All error messages and important status changes of the ChemStation are entered in the logbook.
LCDIAG.REG	For LC only. Contains instrument curves (gradients, temperature, pressures, etc.), injection volume and the solvent descriptions.
ACQRES.REG	Contains column information. For GC it also contains the injection volume.
GLPSAVE.REG	Part of the data file when Save GLP Data is specified.

The method can be stored with the result files. In such cases the method directory is stored as a subdirectory in the data file directory.

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 is only available for ChemStations that support spectra evaluation. It 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 the 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 red triangle. For a Agilent 1100 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, for the Agilent 1100 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.

Color	Status
gray	inactive or off
yellow	not ready
green	ready
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.

Integration

Integration

This chapter describes the following:

- what is integration?
- what does integration do?, and
- the ChemStation integrator algorithms.

What is Integration?

Integration locates the peaks in a signal and calculates their size.

Integration is a necessary step for:

- quantification,
- peak purity calculations (ChemStations for LC 3D, LC/MS Systems and CE systems only), and
- spectral library search (ChemStations for LC 3D, LC/MS Systems and CE systems only).

What Does Integration Do?

When a signal is integrated, the software:

- identifies a start and an end time for each peak and marks these points with vertical tick marks,
- finds the apex of each peak; that is, the retention/migration time,
- constructs a baseline, and
- calculates the area, height and peak width for each peak.

This process is controlled by parameters called integration events.

The ChemStation Integrator Algorithms

The ChemStation includes two integrator algorithms. The standard integrator algorithm was included in earlier versions of the ChemStation and is also included in most other Agilent Technologies analytical data evaluation software. The enhanced integrator algorithm is the first revision of a new generation aimed at improved ruggedness, reliability and ease-of-use. In this software revision we recommend using the traditional algorithm for existing validated methods and the new algorithm for new methods.

A Brief History of the Standard Integrator Algorithm

In the 1980's, the standard integrator algorithm was introduced with the HP 3350 laboratory data system. Later, it was incorporated into the HP 3365 ChemStation system and finally into the new ChemStation.

The standard integrator algorithm was designed to deal with the full range of analytical applications with minimum user optimization.

As the number of new analytical applications has expanded, it was recognized that a single, standard integrator cannot be optimized for all of the conceivable applications of the 1990's without compromising performance.

Compatibility

The standard integrator will continue to be available for those who have already developed method files for the standard integrator, or choose not to migrate to the enhanced integrator at this time. Data files acquired or reanalyzed with the standard integrator can be used with both integrators.

Introducing the Enhanced Integrator Algorithm

Hewlett Packard developed the enhanced integrator as a response to suggestions by customers using the standard integrator in a number of products, who recognized the potential for improved performance. We recognize that an integrator algorithm should be able to take on different *personalities* to deal effectively with the varied analytical techniques in use today or in the future.

Common Integrator Capabilities

Both integrator algorithms include the following key capabilities:

- an autointegrate capability used to set up initial integrator parameters,
- the ability to define individual integration event tables for each chromatographic signal if multiple signals or more than one detector is used,
- interactive definition of integration events that allows users to graphically select event times,
- graphical manual or rubber-band integration of chromatograms or electropherograms requiring human interpretation (these events may also be recorded in the method and used as part of the automated operation),
- display and printing of integration results, and
- the ability to integrate at least 1000 peaks per chromatogram.

Both integrator algorithms include the following groups of commands:

- integrator parameter definitions to set or modify the basic integrator settings for area rejection, peak width and identification threshold (a noise rejection parameter),
- baseline control parameters, such as force baseline, hold baseline, baseline at all valleys, baseline at the next valley, fit baseline backwards from the end of the current peak,
- area summation control,
- negative peak recognition,
- tangent skim processing including solvent peak definition commands, and
- integrator control commands defining retention time ranges for the integrator operation.

Improved Capabilities of the Enhanced Integrator

The enhanced integrator provides the following improved capabilities when compared against the standard integrator:

- optimized baseline tracking using parameters from the individual method and data files,
- additional initial parameters to remove noise generated peaks through the initial peak height parameter,

The ChemStation Integrator Algorithms

- better peak allocation on noisy signals,
- peak shoulder allocation through the use of second derivative or degree of curvature calculations,
- expanded selection of tangent skim calculations which includes straight line, exponential, a calculation that merges these into a smooth transition from exponential to straight line,
- improved sampling of non-equidistant data points for better performance with DAD LC data files that are reconstructed from DAD spectra, and
- ease-of-use — the new integrator algorithm has a new user interface based on tool bars and automatically focusing on key information.

Both integrator algorithms are described in more detail in Chapter 5 “The Standard Integrator Algorithm” and Chapter 6 “The Enhanced Integrator Algorithm”.

Integration

The ChemStation Integrator Algorithms

**The Standard Integrator
Algorithm**

The Standard Integrator Algorithm

This chapter describes the following:

- The standard integrator algorithm,
- integration events,
- ways of integration,
- autointegration,
- integration, and
- manual integration.

The Standard Integrator Algorithm

The standard integrator algorithm was included in earlier versions of the ChemStation and is also included in most other Agilent Technologies analytical data evaluation software. This chapter describes how the standard integrator algorithm works.

When a signal is integrated, the standard integrator algorithm:

- identifies a start and an end time for each peak and marks these points with vertical tick marks,
- finds the apex of each peak; that is, the retention/migration time,
- constructs a baseline, and
- calculates the area, height and peak width for each peak.

This process is controlled by parameters called integration events. The two most important events are threshold and peak width. The ChemStation allows you to set initial values for these and other events. These values are in effect at the beginning of the signal. In most cases you only need these initial events to get good integration results for the entire signal. However, should you wish to time-program the event, use the ChemStation time-programmed events. For further information, see “Integration Events” on page 84.

How Integration Works

The integration process comprises the following:

- peak recognition (cardinal point definition),
- baseline construction, and
- peak area calculation.

Peak Recognition

The first part of integration involves peak recognition, which comprises the following processes:

- finding the start of the peak,
- defining the apex of the peak, and
- finding the end of the peak.

Integration of Isolated Peaks

The integrator scans the digital signal data assuming that the first point of the signal is on the baseline. As the integrator scans the data, it calculates a moving average over a period equivalent to the initial peak width and calls this average the baseline. This process continues as long as the signal curvature remain below a certain value calculated from the threshold value. If this value is exceeded, a peak may be starting, if this continues, the integrator decides that it is on the upslope of a peak. The peak is processed and the integrator returns to the baseline tracking mode.

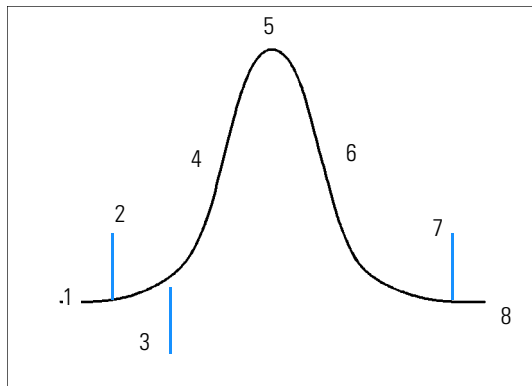
The process for finding a positive peak is as follows (see Figure 2):

- 1 Slope and curvature within limit: track baseline.
- 2 Slope and curvature above limit: possibility of a peak.
- 3 Slope remains above limit: peak recognized.
- 4 Curvature becomes negative: front inflection point.
- 5 Slope becomes negative: apex of the peak.
- 6 Curvature becomes positive: rear inflection point.
- 7 Slope and curvature within limit: approaching end of the peak.
- 8 Slope and curvature remain within limit: end of peak, track baseline.

Peak Recognition

Figure 2

Cardinal Points



Steps 3, 5 and 8 define cardinal points: the start of the peak, the peak apex and the end of the peak respectively.

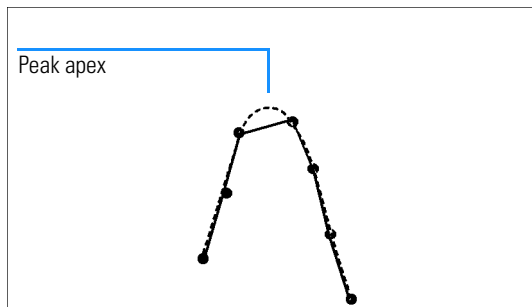
The peak is confirmed if the resulting peak width at half height fits the limits set by the Peak Width option in the integration events. For further details, see “Peak Width” on page 84.

Determining Peak Apex

The slope changes from positive to negative at the top of the peak. To calculate the values for the retention/migration time and peak height, the integrator takes the highest data point and one on either side, fits them to a quadratic equation, and solves the equation to find the highest point.

Figure 3

Determining Peak Apex



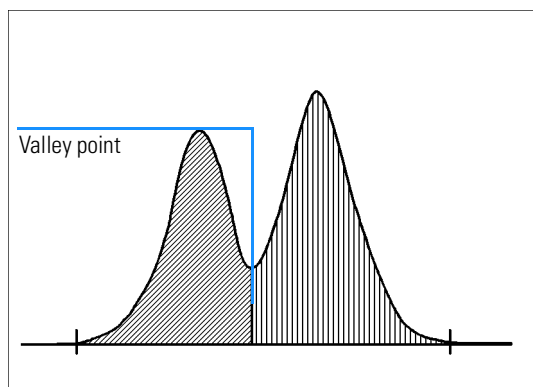
Integration of Merged Peaks

Sometimes two peaks are merged with no baseline between them. In the case of a baseline-valley (BV) and a valley-baseline (VB) peak, the integrator separates the merged peaks by dropping a perpendicular from the valley point between the two peaks. The integrator locates the start of the first peak and begins to sum the area until it locates a valley. At the valley point, area summation from the first peak stops. Area summation for the second peak starts. When the integrator finds the end of the second peak the area summation stops.

See “Peak Separation Codes” on page 95 for more information on peak separation codes.

Figure 4

Merged Peaks

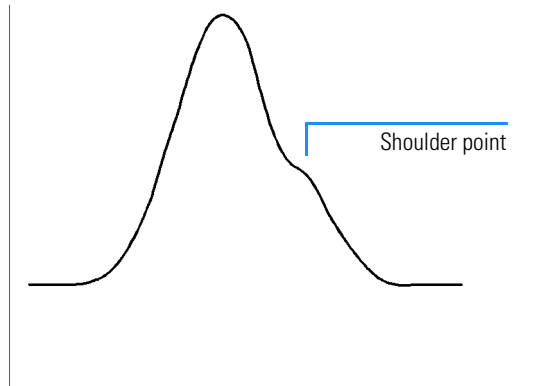


Integration of Shoulders

A shoulder is an unresolved peak on the leading or trailing edge of a larger peak. There is no true valley in the sense of negative slope followed by positive slope.

Figure 5

Peak Shoulders



The shoulders found are reported with their time of maximum negative curvature. The area reported for the main peak includes the area of the shoulders.

Baseline Construction

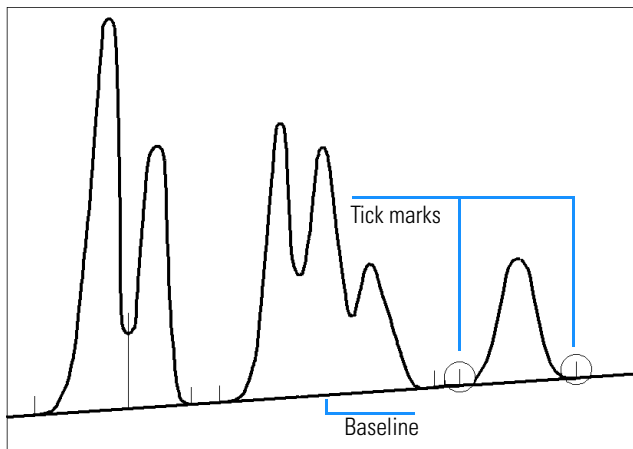
After the peak has been recognized, baseline construction is done to determine the final area of the peak. The baseline construction follows the changes in the signal.

The integrator constructs the baseline as a series of straight-line segments between:

- the signal level at the start of the run,
- the tick-marks (marking the start and end of a peak), and
- the signal level at the end of the run; a point at the stoptime on a horizontal extension from the last declared baseline point.

Figure 6

Default Baseline Construction



Peak Separation Codes

In the reports, each peak is assigned a two letter code that describes how the signal baseline was drawn. If a third letter is present, it provides peak type information. These codes are listed in a column named 'Type' along with the tabular results. The first letter describes the baseline at the start of the peak and the second letter describes the baseline at the end of the peak (separation codes). All codes used in the ChemStation reports are listed in alphabetical order:

A	The peak integration was aborted (peak type).
B	The peak started or stopped on the baseline.
H	The peak started or stopped on the horizontal baseline.
N	This is a negative peak (peak type).
P	The peak started or stopped while the baseline was penetrated.
S	The integrator recognized the peak as solvent peak (peak type).
T	The peak started or stopped while tangent skim (straight line) was enabled (peak type).
V	The peak started or stopped with a valley dropline.
+	The peak is an area summed peak (peak type).
-	The peak is negative, meaning below the baseline (peak type).

If manual integration is used, some other codes may appear as baseline or peak separation codes:

M	The peak was manually integrated.
F	The peak was forced by manual integration. If a peak occurs before the manually integrated peak and the end changes because of manual integration the peak is classified as forced.

Peak Separation Codes

R A solvent peak has been affected by manual integration, such as tangent skim is classified as a re-calculated solvent peak (peak type).

If shoulder detection is ON and shoulders are detected, a four-letter code is written in the Type column:

FSHO A shoulder was detected on the leading edge of the peak.

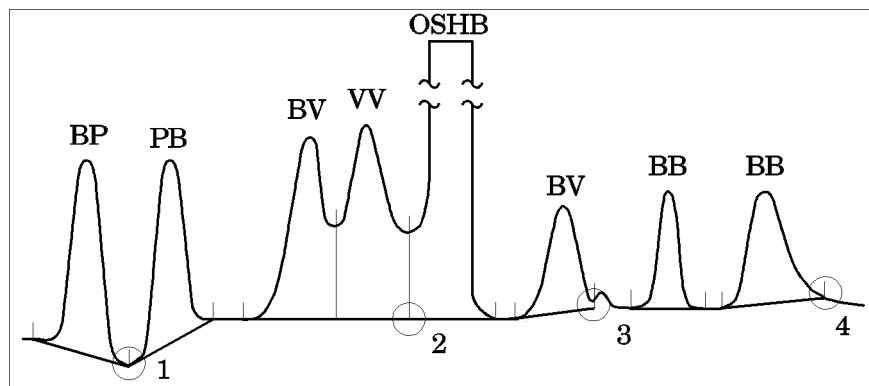
RSHO A shoulder was detected on the trailing edge of the peak.

Modified Baseline Construction

Under certain conditions, the integrator will modify the baseline to include points determined by certain rules, as shown in Figure 7.

Figure 7

Modified Baseline Construction



- If a peak penetrates the baseline (BP and PB), the baseline also goes through the lowest point on the peak, see point 1 in Figure 7.
- If a solvent peak, detected by using tangent skimming does not start on the baseline, then the baseline also goes through a point (see point 2 in Figure 7), on a horizontal extension from the last-declared baseline point to the start of the solvent peak. Overflow solvent horizontal baseline (OSHB) means the signal has exceeded the linear range of the detector.
- If a peak ends in an apparent valley and the following peak is below the area reject, you have set, the baseline is projected from the beginning of the peak to the next true baseline point (see point 3 in Figure 7). If a peak starts in a similar way, the same rule applies.

Peak Separation Codes

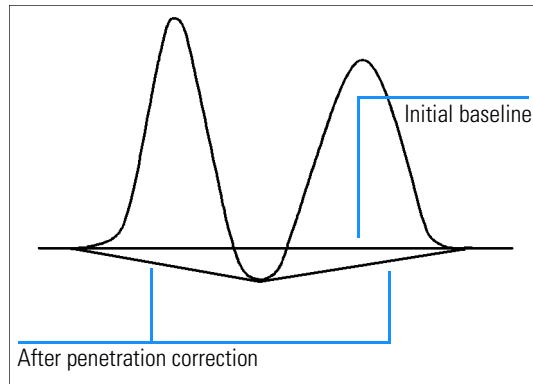
- To improve handling of tailing peaks, the time the peak exceeds upslope and downslope criteria is noted and one quarter of that time is added at the end of this peak before the end of the peak is confirmed (see point 4 in Figure 7).

Baseline Penetrations

A penetration occurs when the signal drops below the constructed baseline. If a baseline penetration occurs, that part of the baseline is redrawn.

Figure 8

Baseline Penetration



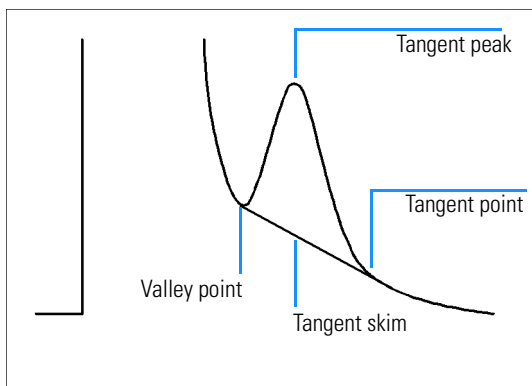
Tangent Skimming

Tangent skimming is a form of baseline construction done for peaks that are found on the downslope of any peak designated as a solvent peak.

The integrator uses a calculation to draw a tangent from the valley before the rider peak of the solvent peak to a tangent point after the tangent peak (rider peak) of the solvent peak where the detector signal slope is equal to the tangent slope (see Figure 9).

Figure 9

Tangent Skimming



Peak Area Measurement

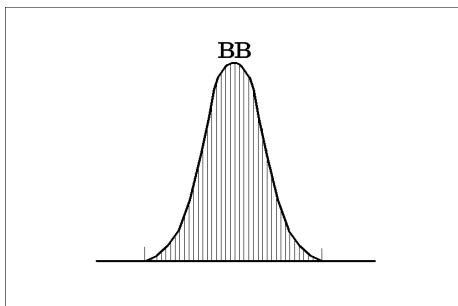
The last step in peak integration is determining the final area of the peak.

The area which the integrator calculates during integration is determined as follows:

- for baseline-to-baseline (BB) peaks, the area is above the baseline between the tick marks

Figure 10

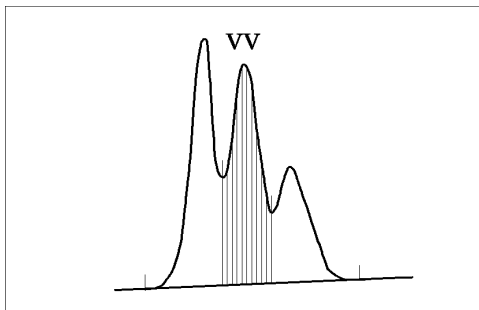
Area Measurement for Baseline-to-Baseline Peaks,



- for valley-to-valley (VV) peaks, the area above the baseline is divided with vertical dropped lines from tick marks,

Figure 11

Area Measurement for Valley-to-Valley Peaks



- for tangent peaks, the area is above the reset baseline,
- for solvent peaks, the area is above the horizontal extension from the last-found baseline point and below the reset baseline given to tangent peaks. A solvent peak may rise too slowly to be recognized, or there may

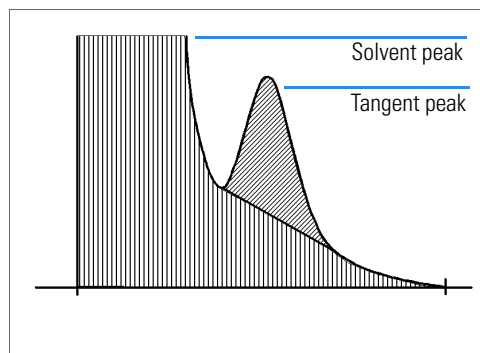
Peak Area Measurement

be a group of peaks well into the run which you feel should be treated as a solvent with a set of riders. This usually involves a merged group of peaks where the first one is far larger than the rest. The simple dropline treatment would exaggerate the later peaks because they are actually sitting on the tail of the first one. By forcing the first peak to be recognized as a solvent, the rest of the group is skimmed off the tail.

Although a solvent peak is not applicable to CE instruments, it is possible that the label also appears for large peaks in the ChemStation for CE systems.

Figure 12

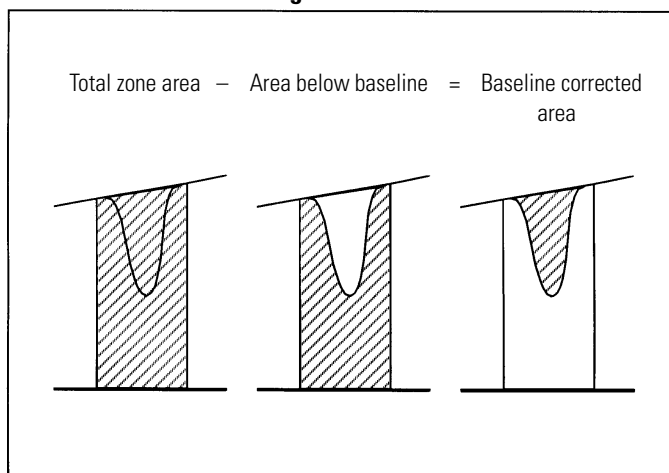
Area Measurement for Tangent and Solvent Peaks



- Negative peaks that occur below the baseline, have a positive area as shown in Figure 13.

Figure 13

Area Measurement for Negative Peaks



Integration Events

There are four initial integrator events and nineteen timed integrator events. Many events are on and off or start and stop pairs.

Initial events

Initial Peak

Width Sets the initial width of the peak at half height.

Initial

Threshold Sets a minimum signal height value for peak rejection.

Initial Area

Reject Sets an area value below which all peaks are rejected.

Shoulder

Detection Enables (on) or disables (off) shoulder detection.

Peak Width

The standard integrator calculates the peak width W using the following formula (see Figure 14 for details):

$$W = 0.3 \times (t_2 + t_3) + 0.7 \times \frac{A}{H}$$

where:

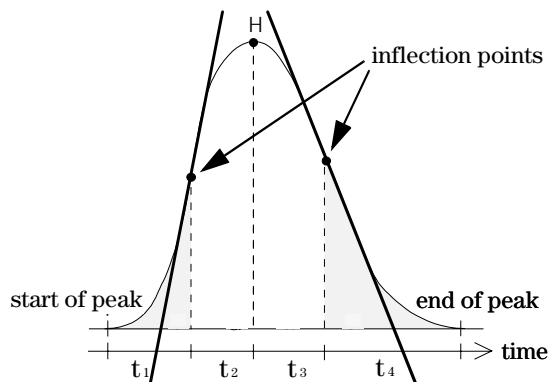
t_i = time slice

A = peak area

H = peak height

This is the same as the width at half height for a Gaussian peak where $t_2 + t_3$ and A/H are identical. If the peak has any tailing, it will show a larger width than the width at half height, so that when used to calculate theoretical plates the number of plates for tailing peaks will be underestimated. However, the integrator will continue to give acceptable values for peak width when peaks are badly merged.

Figure 14

Peak Width Calculation

The peak width setting controls the selectivity of the integrator to distinguish peaks from baseline noise. To choose a suitable peak width for a single peak of interest, use the peak's time width in minutes at half height.

To use the peak width successfully, the best setting should be chosen which provides just enough filtering to prevent noise being interpreted as peaks but not enough to distort the signal information. If the selected peak width is too low, noise may be interpreted as peaks.

The initial peak width should be the width of the narrowest peak. Typically in an isocratic/isothermal run, peak widths broaden as the run progresses. In such cases it may be necessary to change the peak width in the Integration Events dialog box during a run to match changing peak widths. It may happen that broader and narrower peaks are mixed. The peak width can be time-programmed for different peaks by using the peak width event in the Integration Events dialog box.

The peak width is automatically updated during the integration process as the peaks are identified. The update is weighted as follows:

$$0.75 \times \text{existing peak width} + 0.25 \times \text{width of current peak}$$

The update is limited to a change of 25%. If a timed integration event is used during the integration process, the automatic peak width adjustment is disabled.

Peak Width Range

The peak width range is the range of peak widths within which a peak can be detected. If a peak has a peak width that is above or below the limits of the peak width range, then the peak is *not* detected, see Figure 15. The size of the

Integration Events

peak width range depends on the signal-to-noise ratio of the signal. If there is a good signal-to-noise ratio, for example, 100:1 or above, then the lower limit of the peak width range will be about one third of the specified peak width and the upper limit will be about three times that of the specified peak width value. If there is a bad signal-to-noise ratio, for example, 5:1, then the peak width range will be reduced to approximately 90 % to 110 % of the specified peak width value.

Threshold

The threshold sets the noise rejection for integration.

The threshold value determines a minimum signal height value for integration. For example, peaks smaller in height than the threshold will be ignored by the integrator. The higher the threshold setting, the greater the height above the established baseline must be before a data point qualifies as the start of a peak.

The threshold is calculated using the following formula:

$$\text{Threshold} = c2^{n-6}$$

where:

c is a detector dependent constant, and

n is the threshold value.

The threshold value is based on a relative scale ranging from -20 to 25. The following table shows the relationship between the threshold setting and mAU for the detectors that can be used with the ChemStation. The detectors are the HP 1090 diode array detector, the Agilent CE diode array detector, the HP 1046 fluorescence detector, the HP 1050 diode array detector, the HP 1050 Series variable wavelength detector and the HP 1050 Series multiple wavelength detector. The same values apply to the Agilent 35900C/D/E dual channel interface (analog-to-digital converter) in mV.

The threshold should not be used to reject peaks whose heights approach that of the smallest peaks of interest because the threshold is not designed to do this. To exclude small peaks the area reject event is recommended.

Area Reject

The area reject sets peak rejection by final area. Any peaks that have areas less than the minimum area will not be reported.

Table 1

Threshold Values for LC Detectors

Threshold Setting	LC Detector (mAU) DCI (mV)
-7	0.03125
-6	0.0625
-5	0.125
-4	0.25
-3	0.5
-2	1
-1	2
0	4
1	8
2	16
3	31
4	62
5	125
6	250
7	500
8	1000

Shoulder Detection

When the shoulder detection is on, the integrator uses an extended peak-integration algorithm that can identify shoulders on a peak.

Changing Threshold and Peak Width

Both peak width and threshold are very important in the integration process and by changing the values you can achieve different results.

- Increasing both threshold and peak width are useful where relatively dominant components must be detected and quantified in a high noise environment. An increased peak width increases the signal filtering and an increased threshold ensures that random noise is ignored.
- Decreasing threshold and peak width are useful in detecting and quantifying trace components, whose heights approach that of the noise level itself. Decreasing peak width gives decreased signal filtering, while decreasing threshold ensures that small peaks are not rejected because they have insufficient height.
- In a situation where peaks exhibit a mix of widths, and both broad and

Integration Events

narrow ones must be detected and quantified without changing peak width, peak width should be set for the more narrow peaks, and threshold reduced to ensure that broader peaks are still detected.

Customizing Integration

It is often useful to change the values for the peak width, threshold and area reject to customize the integration of your signal.

Figure 15 shows the influence of threshold, peak width and area reject on the integration of five peaks in a signal.

Figure 15

Using Initial Events

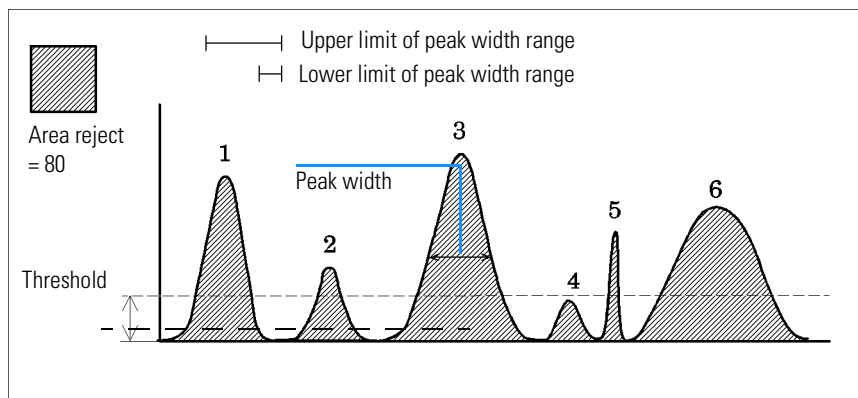


Table 2

Area Values

Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
110	50	200	32	30	241

Integration Events

A peak is only integrated when all three integration event requirements are satisfied. Using the peak width for peak 3, the area reject and threshold shown only in peaks 1 and 3 will be integrated.

Table 3

Threshold Values						
Integration Event	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
Threshold	Above	Above	Above	Below	Above	Above
Peak width range	Within	Within	Within	Within	Below	Above
Area reject	Above	Below	Above	Below	Below	Above
Peak integrated	Yes	No	Yes	No	No	No

Peak 1 is integrated as all three integration event requirements are satisfied.

Peak 2 is rejected because the area is below the set area reject value.

Peak 3 is integrated as all three integration event requirements are satisfied.

Peak 4 is not integrated because the peak height is below the set threshold.

Peak 5 is not integrated because the peak width value is lower than the lower limit of the peak width range.

Peak 6 is not integrated because the peak width value exceeds the upper limit of the peak width range.

Timed Events

You can use timed integrator events to customize signal baseline construction when default construction is not appropriate. These events can be useful in summing of final peak areas and correcting for short and long term baseline aberrations. For further information on integration events see your *online help*.

Integration Events Tables

Each signal (chromatogram or electropherogram) acquired in a particular method may have its own integration parameters set up in the signal integration events table. These tables are not used unless the user specifically sets them up by either

- selecting them and defining events in the integration events dialog box, or
- selecting autointegrate that optimizes the events for each signal.

If the individual signal events table is not used all signals use the default integration events table

Integration Methods

The ChemStation provides the following integration methods:

- autointegration,
- integration, and
- manual integration.

Autointegration

AutoIntegrate examines the beginning and end regions of the chromatogram to estimate noise and assigns appropriate initial values of Threshold and Area Reject. AutoIntegrate also determines a **temporary** value for Peakwidth based on the run time and minimum detection criteria. With the Area Reject set to zero, a trial integration is performed.

If no peaks are detected in the trial integration, the parameters are adjusted and the trial is repeated, several times if necessary. Widths from the earliest peak found are combined to produce an initial Peakwidth value which AutoIntegrate uses for the autointegrated chromatogram.

Limitations

AutoIntegrate is a useful starting point for most chromatograms and in the early stages of method development. AutoIntegrate should not, however, be used or accepted as a substitute for good sample preparation and chromatographic techniques.

AutoIntegrate determines values for the **initial time** of the run which limits AutoIntegrate's ability to deal with unusual situations that may occur *later* in the chromatogram.

AutoIntegrate may have problems dealing with chromatograms having disturbances or peaks at the start or the end of the run.

These kinds of disturbances include:

- Inverted peaks
- Baseline upsets and transients (valve or signal switched)
- Rising baseline at the end of the run (column bleed)

In cases where AutoIntegrate alone does not give fully satisfactory results, you can program the integration events. In the chromatogram above, the following integration timed events are used.

Integrator Off

Integrator On, Baseline All Valleys

Set Baseline

Integrator timed events to change the detection parameters may also be necessary.

Integration

The signal is integrated according to the values in the integration events table. You can use this function to adjust the integration results by changing the integration events, and then reintegrating the signal.

Both `autointegrate` and `integrate` provide integration results. However, `autointegration` automatically calculates the initial events of threshold and peak width, whereas the Edit Integration event function allows you to specify these integration events or use the default values.

Manual Integration

This type of integration allows you to integrate selected peaks or groups of peaks. Except for the initial area reject value, the ChemStation's event integration is ignored within the specified range of manual integration.

Manual integration allows you to define the peak start and stop points, and then include the recalculated areas in the quantification and reporting packages. Manually integrated peaks in the reports are annotated using the peak separation code M.

Manual Integration offers the following features:

- Draw Baseline** Specifies where the baselines are to be drawn for a peak or set of peaks. You can also specify whether peaks in the range given should be automatically separated at all valley points.
- Negative Peaks** Specifies when to treat any areas below the baseline as negative peaks. You can also specify whether peaks in the range given should be automatically separated at all valley points.
- Tangent Skim** Calculates the areas of peaks tangentially skimmed off a main peak. You can also specify whether peaks in the range given should be automatically separated at all valley points. The area of the tangent skimmed peak is subtracted from the area of the main peak.
- Split Peak** Specifies a point where to split a peak with a dropline.
- All Valleys** All Valleys is a menu item that can be active or inactive. When active, peaks will automatically be separated at all valleys within the range of manual integration when one of the menu items Draw Baseline, Negative Peaks or Tangent Skim is used.
- Trace Mode** Activates a vertical arrow cursor that cannot be moved off the signal. This ensures that both the start and end points of the peaks lie on the chromatogram/electropherogram.
- Delete Peak(s)** Deletes one or more peaks from the integration results.

Peak Separation Codes

- Manually integrated peaks are reported in the integration reports by the peak code MM.
- If there is a peak before the manually integrated peak and the end of this peak changes because of the manual integration, it will have a code F (for forced).
- A solvent peak which has been affected by manual integration, such as tangent skim, will be labeled with an R (for recalculated solvent) instead of an S.

Manual Integration Process

- The first step in manual integration is to select an area of the signal to integrate manually. This is done by zooming into the area of interest.
- The next step is to choose Draw Baselines, Negative Peaks, Tangent Skim or Delete Peak(s).
- The baselines can now be drawn by dragging the mouse while holding down the left mouse button. As the mouse is moved, a line is drawn from the starting point to the point where the mouse is currently positioned. In the case of Delete Peak(s) a rectangle is drawn around the peaks to be deleted.
- The graphical input operates in two modes: Absolute (default) and Trace. In both modes the message line displays the time and level of the cursor.
- In Trace mode, the cursor is an arrow pointing downwards, which follows the shape of the signal, by moving from data point to data point. You can activate the Trace mode when the cursor is positioned at the desired start of a peak, by pressing the right mouse button.
- In Absolute mode, the cursor is a crosshair and does not follow the signal but allows you to position the cursor anywhere on the screen. You can move from one mode to the other by clicking the right mouse button.
- For each peak integrated manually, the area counts will be displayed with the retention/migration time label on the screen.

Copying Manual Events to the Method

The Copy Manual Events to Method item from the Integration menu allows you to copy the integration events defined by manual integration to the

Manual Integration

currently loaded method. These manual events are then stored separately in the method. They cannot be reviewed in the Integration Events dialog box. When a signal is loaded they are only applied, if the Apply Manual Integration Events option is checked in the Integration Events dialog box or when you select Apply Manual Events from Method in the Integration Menu.

The manual integration events use absolute time values. They do not adjust for signal drift.

**The Enhanced
Integrator Algorithm**

The Enhanced Integrator Algorithm

This chapter describes the following:

- The enhanced integrator,
- how is the enhanced integrator customized for my configuration?
- an overview of how the enhanced integrator works,
- integrating peaks in the real world,
- more about the enhanced integrator,
- baseline allocation,
- peak separation codes,
- tangent skimming of peaks,
- peak area measurement,
- integration events, and
- using the enhanced integrator.

The Enhanced Integrator Algorithm

The enhanced integrator algorithm, a new generation integrator aimed at improved ruggedness, reliability and ease-of-use, was introduced at the A.04.01 revision of the ChemStation. In this software revision we recommend using the standard algorithm for existing validated methods and the enhanced algorithm for new methods.

How is the Enhanced Integrator Customized for my Configuration?

The enhanced integrator is designed to be customized for optimum performance by using:

- fixed integration parameters, and
- adaptive integration parameters.

Fixed Integration Parameters

The fixed integration parameters are *fixed* in the sense that they are not accessible from the normal user interface. Most of these parameters are set by Agilent Technologies for a particular hardware/software configuration. However, some are set by information obtained by the method and data files.

To illustrate how this works, consider that different chromatographic systems may require that the calculation of the peak apex be handled in a certain way. Agilent Technologies can configure the enhanced integrator to calculate the peak apex using one of several ways. It can use the highest data point, a quadratic fit, the first moment, or a Gaussian fit. The appropriate calculation type is chosen to optimize the integrator for the particular hardware (detector, column type, and so on) and the application.

We chose the peak apex calculation to illustrate the concept of the fixed integration parameters. There are actually more than a dozen additional fixed parameters. Though these parameters should be considered fixed by most users, advanced users can access them by writing the appropriate macro as described in the *Macro Programming Guide*.

Adaptive Integration Parameters

You will recognize the adaptive integration parameters as those that are user accessible.

The adaptive integrator parameters include:

- integration parameters set by the user before a run (Area Reject, Initial PW, and so on), and
- parameters set by several integrator timed events (Clamp Signal).

How Does the Enhanced Integrator Work?

When you break down the integration process into its most fundamental steps, the enhanced integrator must work the same as any integrator. How the integrator performs these steps determines how accurate and reproducible your results will be.

The integrator performs the following tasks when integrating a peak:

- defines the initial baseline,
- continuously tracks and updates the baseline,
- identifies a start time for the peak, and marks this point with a vertical tick mark,
- finds the apex of each peak and prints the retention/migration time,
- identifies an end time for the peak, and marks this points with a vertical tick mark,
- constructs a baseline, and
- calculates the area, height, and peak width for each peak.

This process is controlled by parameters called integration events. The most important events are called initial slope sensitivity, peak width, and height reject. The ChemStation allows you to set initial values for these and other events. These values are in effect at the beginning of the signal.

In most cases, these initial events are all that are needed to get good integration results for the entire signal. However, there may be time when you wish to perform an integration in a certain way.

You can control how an integration is performed by:

- using the ChemStation time programmed events, and
- using a macro program to customize the integrator operation.

For more information, see the fixed parameter set for the enhanced integrator in the *Macro Programming Guide* which is available as online help.

Defining the Initial Baseline

The integrator uses parameters from the method and the data file to optimize the baseline for the particular application.

Before the integrator can begin integrating peaks, it tries to define a baseline point. When the analysis begins, the integrator establishes an initial baseline level by taking the first data point as a tentative *baseline point*. It then attempts to redefine the initial baseline point based on the average of the input signal. If the integrator does not obtain a redefined initial baseline point, it retains the first data point as a potential initial baseline point.

Baseline Tracking

The integrator samples the digital data at a rate determined by the initial peak width or by the calculated peak width, as the run progresses. It considers each data point as a potential baseline point.

To become a baseline point, a data point must satisfy the following conditions:

- the data point must lie within the defined baseline envelope, and
- the curvature of the baseline at the data point (determined by the derivative filters), is below a critical value, as determined by the current slope sensitivity setting.

The integrator determines a *baseline envelope* from the slope of the baseline using a baseline tracking algorithm where the slope is determined by the first derivative and the curvature is determined by the second derivative.

The initial baseline point, established at the start of the analysis is then continuously reset, at a rate determined by the peak width, to the *moving average* of the data points that lie *within the baseline envelope* over a period determined by the peak width. The integrator tracks and periodically resets the baseline to compensate for drift, until a peak upslope is detected as shown in Figure 16.

Allocating the Baseline

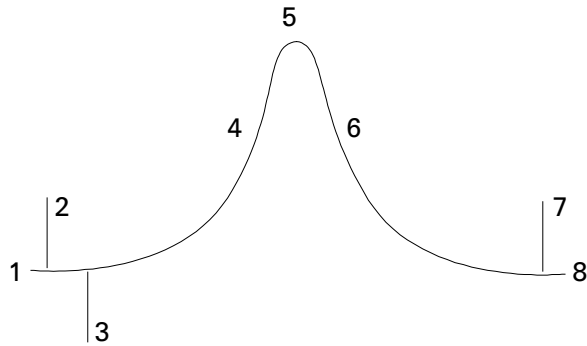
The integrator allocates the chromatographic baseline periodically during the analysis. The peak width value determines the frequency of baseline allocation. When the integrator has sampled a certain number of data points, it resets the baseline from the initial baseline point to the current baseline point. The integrator resumes tracking the baseline over the next set data

How Does the Enhanced Integrator Work?

- 8 Slope and curvature remain within limit: end of peak, cardinal point defined.
- 9 The integrator returns to the baseline tracking mode.

Figure 17

Integrated Peak



Cardinal points are the points chosen by the integrator to define and quantify a peak. Baseline points, valley points, peak apex, and points of inflection are designated cardinal points and saved. Each cardinal point has a horizontal coordinate of elapsed time, a vertical coordinate of height from the baseline, and other parameters the integrator uses to calculate the peak areas.

Integrating Peaks in the Real World

In the real world, the integrator typically must deal with more complicated chromatographic problems. The size of peaks can vary tremendously within a single run, while the peaks of interest are often present in small concentrations. Interferences such as system noise, drift, and wander can affect the baseline from which the integrator measures peak area and height. Finally, it may not be possible to separate peaks completely with the chromatographic process available. Whenever possible, the chromatography should be optimized to produce separation. When this is not possible for some reason, the integrator must deal with overlapping peaks.

So we see that peak integration is a complicated task. While the enhanced integrator cannot fully compensate for poor chromatography, it can overcome noise, drift, and incomplete peak separations to obtain reproducible results from difficult chromatograms.

More About the Enhanced Integrator

Now let's examine in detail how the integrator recognizes the start of a peak, the peak apex, and the end of peak, how it locates the cardinal points for baseline allocation, and the calculation of areas.

The integration process comprises the following:

- peak recognition (cardinal point definition),
- baseline allocation, and
- peak area measurement.

Peak Recognition

The enhanced integrator uses several *tools* to recognize a peak:

- peak width,
- peak recognition filters,
- bunching,
- peak recognition algorithm,
- peak apex algorithm, and
- non-Gaussian calculations (tailing, merged peaks, and so on).

Peak Width

The peak width setting controls the selectivity of the integrator to distinguish peaks from baseline noise. To obtain good performance, the peak width must be set close to the width of the actual chromatographic peaks.

There are three ways the peak width is changed:

- before the run, the user can specify the initial peak width,
- during the run, the integrator automatically updates the peak width as necessary to maintain a good match with the peak recognition filters, or
- during the run, the peak width can be set or modified with a time programmed event.

Peak Recognition Filters

The integrator uses three peak recognition filters as one of the tools for recognizing peaks by detecting changes in the slope and curvature within a set of contiguous data points. These filters contain the first derivative (to measure slope) and the second derivative (to measure curvature) of the data points being examined by the integrator. The recognition filters are defined below:

Filter 1	Slope and curvature values of two contiguous data points
Filter 2	Slope and curvature values of four contiguous data points
Filter 3	Slope and curvature values of eight contiguous data points

Peak Recognition

The actual filter used is determined by the peak width setting. For example, at the start of an analysis, Filter 1 may be used. If the peak width increases during the analysis, Filter 2 and eventually Filter 3 would be used. To obtain good performance from the recognition filters, the peak width must be set close to the width of the actual chromatographic peaks. The integrator will update the peak width as necessary during the run to optimize the integration.

The enhanced integrator can calculate the peak width for updating in several ways, depending on the instrument:

For LC/CE configurations, the enhanced integrator calculates peak width by default using a composite calculation

$$(0.3 \times (\text{Right inflection point} - \text{Left inflection point}) + 0.7 \times \text{Area/Height}).$$

For GC configurations, the enhanced integrator calculates peak width by default as area/height. This calculation will not overestimate the width when peaks are merged above the half-height point.

In certain types of analyses, peaks may become significantly broader as the analysis progresses. Isothermal GC and isocratic LC analyses have this characteristic. To compensate for this, the integrator automatically updates the peak width as peaks broaden during an analysis. It does this automatically unless it has been disabled or set to a specific value with a timed event.

The Peak Width update is weighted in the following way:

$$[0.75 \times (\text{existing peak width}) + 0.25 \times (\text{width of current peak})]$$

If a timed integration event disables or sets the peak width to a specific value, the automatic peak width adjustment is disabled.

Bunching

The integrator cannot continue increasing the peak width for broadening peaks indefinitely. Eventually the peaks would become so broad that they could not be *seen* by the peak recognition filters. To overcome this limitation, the integrator uses *bunching*. By bunching the data points together, the integrator effectively narrows the peak while maintaining the same area. Bunching then, is how the integrator keeps broadening peaks within the effective range of the peak recognition filters to maintain good selectivity.

When it is enabled, bunching is performed in the powers of two based on the expected or experienced peak width. The bunching algorithm is summarized in Table 4.

Peak Recognition**Table 4**

Bunching Criterion		
Expected Peak Width (T)	Filters Used	Bunching Done
0 – 10 Data Points	First	None
8 – 16 Data Points	Second	None
12 – 24 Data Points	Third	None
16 – 32 Data Points	Second	Once
24 – 48 Data Points	Third	Once
32 – 96 Data Points	Third, second	Twice
64 – 192 Data Points	Third, second	3 times

The Peak Recognition Algorithm

The integrator identifies the start of the peak with a baseline point determined by the peak recognition algorithm. The peak recognition algorithm first compares the outputs of the peak recognition filters to the value of the initial slope sensitivity to increase or decrease the upslope accumulator. When its value is ≥ 15 , the integrator declares that point a point to indicate that a peak has begun.

The peak recognition algorithm is summarized in Table 5 and Table 6.

Table 5

Incremental Values to Upslope Accumulator			
Derivative Filter 1-3 Outputs Against Slope Sensitivity	Filter 1	Filter 2	Filter 3
Slope > Slope Sensitivity	+ 8	+ 5	+ 3
Curvature > Slope Sensitivity	+ 0	+ 2	+ 1
Slope < (-) Slope Sensitivity	- 8	- 5	- 3
Slope < Slope Sensitivity	- 4	- 2	- 1
Curvature < (-) Slope Sensitivity	- 0	- 2	- 1

In Table 5 the expected peak width determines which filter's slope and curvature values are compared with the Slope Sensitivity. For example,

Peak Recognition

Filter 1 numbers are added to the upslope accumulator when the expected peak width is small. If the expected peak width increases, then the numbers for Filter 2 and, eventually Filter 3 are used.

When the value of the upslope accumulator is greater than or equal to 15, the algorithm recognizes that a peak may be starting.

Table 6**Incremental Values for Downslope Accumulator**

Derivative Filter 1-3 Outputs Against Slope Sensitivity	Filter 1	Filter 2	Filter 3
Slope > Slope Sensitivity	+ 8	+ 5	+ 3
Curvature > Slope Sensitivity	+ 0	+ 2	+ 1
Slope < (-) Slope Sensitivity	- 11	- 7	- 4
Slope < Slope Sensitivity	- 28	- 18	- 11
Curvature < (-) Slope Sensitivity	- 0	- 2	- 1

In Table 6 the expected peak width determines which filter's slope and curvature values are compared with the Slope Sensitivity. For example, Filter 1 numbers are added to the downslope accumulator when the expected peak width is small. If the expected peak width increases, then the numbers for Filter 2 and, eventually Filter 3 are used.

When the value of the downslope accumulator is greater than or equal to 15, the algorithm recognizes that a peak may be ending.

The Peak Apex Algorithm

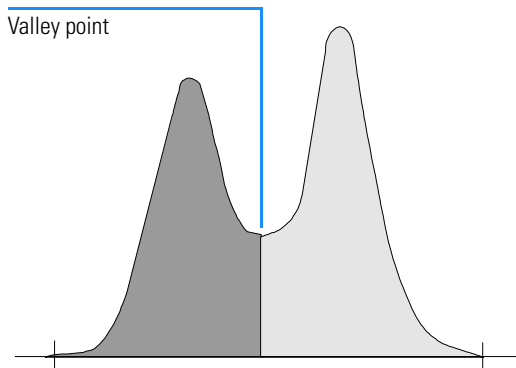
The peak apex is recognized as the high points in the chromatogram by constructing a parabolic fit that passes through the highest data points.

Non-Gaussian Calculations

Merged peaks Occurs when a new peak begins before the end of peak is found. The figure below illustrates how the integrator deals with merged peaks.

Figure 18

Merged Peaks



The integrator processes merged peaks in the following way:

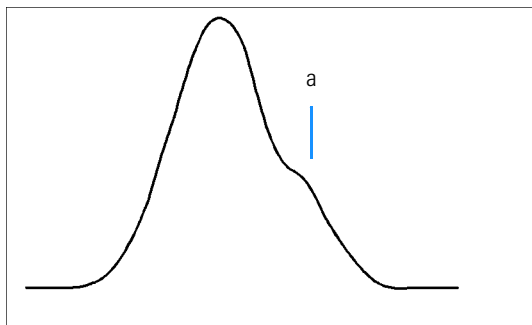
- 1 The integrator sums the area of the first peak until the valley point.
- 2 At the valley point, area summation for the first peak ends and summation for the second peak begins.
- 3 When the integrator locates the end of the second peak, the area summation stops. This process can be visualized by separating the merged peaks by dropping a perpendicular from the valley point between the two peaks.

Peak Recognition

Shoulders Are unresolved peaks on the leading or trailing edge of a larger peak. When a shoulder is present, there is no true valley in the sense of negative slope followed by positive slope. A peak can have any number of front and/or rear shoulders.

Figure 19

Peak Shoulders



Shoulders are detected from the curvature of the peak as given by the second derivative. When the curvature goes to zero, the integrator identifies a point of inflection like point “a” in Figure 19.

- A potential front shoulder exists when a second inflection point is detected before the peak apex. If confirmed, the start of the shoulder point is set at the maximum positive curvature point before the point of inflection.
- A potential rear shoulder when a second inflection point is detected before the peak end or valley. If confirmed, the start of the shoulder point is set at the target point from starting point to curve.

Retention time is determined from the shoulder’s point of maximum negative curvature. With a programmed integration event, the enhanced integrator can also calculate shoulder areas as normal peaks with droplines at the shoulder peak points of inflection.

The area of the shoulder is subtracted from the main peak.

Peak shoulders can be treated as normal peaks by use of an integrator timed event.

Baseline Allocation

After any peak cluster is complete, and baseline is found, the integrator requests the baseline allocation algorithm allocates the baseline using a *pegs-and-thread* technique. It uses trapezoidal area and proportional height corrections to normalize and maintain the lowest possible baseline. Inputs to the baseline allocation algorithm also includes parameters from the method and data files that identify the detector and the application which the integrator uses to optimize its calculations.

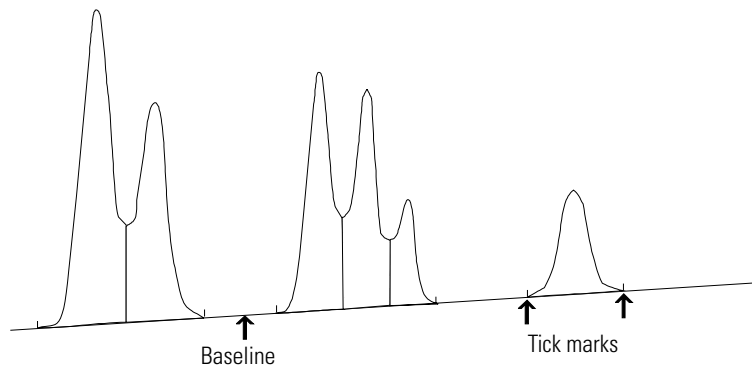
Default Baseline Construction

In the simplest cases, the integrator constructs the baseline as a series of straight line segments between:

- The start of baseline,
- The tick marks, and
- The end of peak.

Figure 20

Default Baseline Construction



Start of Baseline

If no baseline is found at the start of run, the start of baseline will be established in one of the following ways:

- From the start of run to the first baseline point, if the start of run point is

Baseline Allocation

lower than the first baseline point.

- From the start of run to the first valley point, if the start of run point is lower than the first valley.
- From the start of run to the first valley point, if the first valley penetrates an imaginary line drawn from the start of run to the first baseline.
- From the start of run to a horizontal baseline extended to the first baseline point.

Tick Marks

Tick marks identify the beginning and ending of a peak. Their position is determined by the peak start and peak end time saved in the peak table.

End of Baseline

The last valid baseline point is used to designate the end of baseline. In cases where the run does not end on the baseline, the end of baseline is calculated from the last valid baseline point to the established baseline drift.

Peak Separation Codes

In the reports, each peak is assigned a two letter code that describes how the signal baseline was drawn. If a third letter is present, it provides peak type information. These codes are listed in a column named 'Type' along with the tabular results. The first letter describes the baseline at the start of the peak and the second letter describes the baseline at the end of the peak (separation codes). All codes used in the ChemStation reports are listed in alphabetical order:

A	The peak integration was aborted (peak type).
B	The peak started or stopped on the baseline.
H	The peak started or stopped on the horizontal baseline.
N	This is a negative peak (peak type).
P	The peak started or stopped while the baseline was penetrated.
S	The integrator recognized the peak as solvent peak (peak type).
T	The peak started or stopped while tangent skim (straight line) was enabled (peak type).
U	Marks the beginning or end of an unassigned peak.
V	The peak started or stopped with a valley dropline.
X	Exponential tangent skim (peak type).
+	The peak is an area summed peak (peak type).

If manual integration is used, some other codes may appear as baseline or peak separation codes:

M	The peak was manually integrated.
F	The peak was forced by manual integration. If a peak occurs before the manually integrated peak and the end changes because of manual integration the peak is classified as forced.
R	A solvent peak has been affected by manual integration, such as tangent skim is classified as a re-calculated

Peak Separation Codes

solvent peak (peak type).

If shoulder detection is ON and shoulders are detected, the peak separation code will contain one of the following letters in columns three or four:

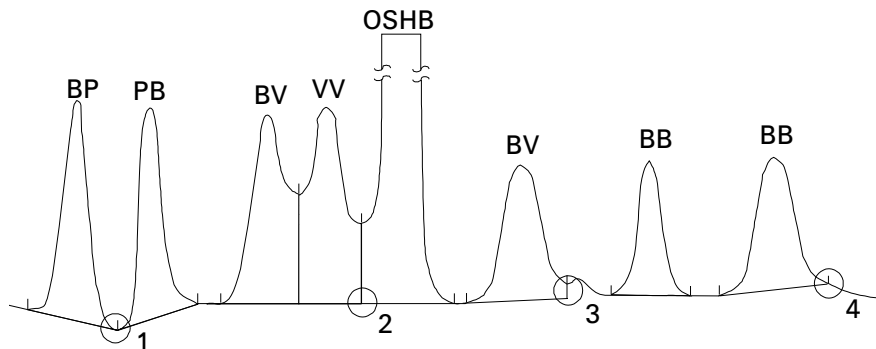
F	A front shoulder was detected—find area with droplines.
B	A rear shoulder was detected—find area with droplines.
f	A front shoulder was detected—tangent skim the area.
b	A rear shoulder was detected—tangent skim the area.

If a fourth letter is present, it provides additional peak information (peak flag). An *O* in the peak code indicates that an over-range condition has occurred, *U* means ‘under-range’ and *D* ‘distorted’.

Modified Baseline Construction

Most analyses produce a more complex chromatogram like that shown in Figure 21. The integrator modifies the baseline using the following rules:

Figure 21

Modified Baseline Construction

- If a peak penetrates the baseline (BP and PB), the baseline also goes through the lowest point on the peak, see point 1 in Figure 21.
- If a solvent peak detected using tangent skimming does not start on the baseline, the baseline also goes through a point, see point 2 in Figure 21, on a horizontal extension from the last-declared baseline point to the start of the solvent peak. Overflow solvent horizontal baseline (OSHB) means the signal has exceeded the linear range of the detector.
- If a peak ends in an apparent valley but the following peak is below area reject, as you have set it, the baseline is projected from the beginning of

Peak Separation Codes

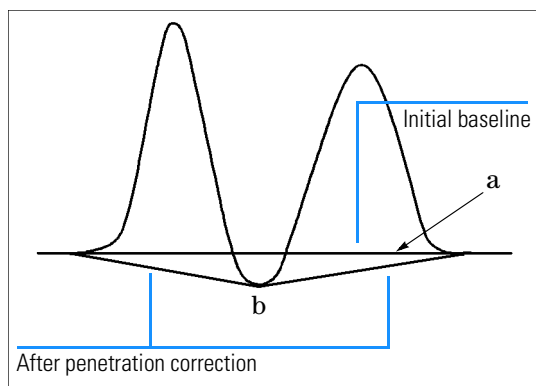
the peak to the next true baseline point, see point 3 in Figure 21. If a peak starts in a similar way, the same rule applies.

Baseline Penetrations

A penetration occurs when the signal drops below the constructed baseline, point “a”. If a baseline penetration occurs, that part of the baseline is redrawn as shown by point “b”.

Figure 22

Baseline Penetration



Tangent Skimming

Tangent skimming is a form of baseline construction done for peaks found on the downslope of a solvent peak. When it is enabled by a user-programmed timed event, a solvent peak can be tangent skimmed in the following ways:

- straight line calculation,
- exponential calculation, and
- combine exponential and straight line calculations for the best fit (default).

The appropriate tangent skim calculation is chosen for a particular application when the ChemStation software is configured. By default, the method chosen is to combine exponential and straight line calculations for the best fit. The standard integrator algorithm uses the same calculation.

Peak Separation Codes

The switch from an exponential to a linear calculation is performed in a way that eliminates abrupt discontinuities of height or areas.

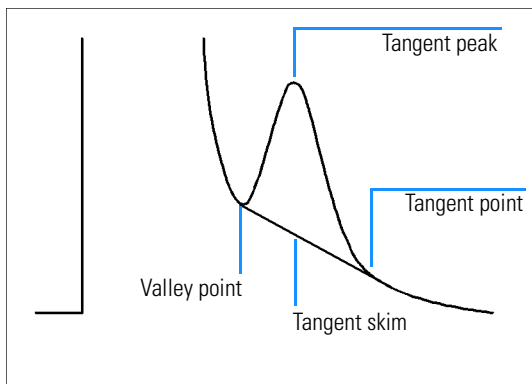
- When the signal is well above the baseline, the tail-fitting calculation is exponential.
- When the signal is within the baseline envelope, the tail fitting calculation is a straight line.

The combination calculations are reported as *exponential* or *tangent skim*.

When a tangent skim is runtime programmed, the integrator compares the signal to the tangent point. The tangent point is found by making an adjustment to the valley point of the tangent peak as shown in Figure 23.

Figure 23

Tangent Skim

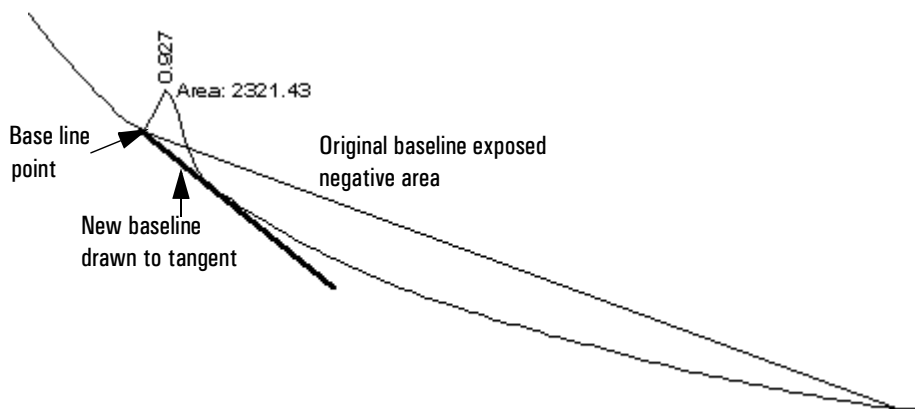


Sometimes, particularly when tangent skimming small peaks on a solvent tail, the usual baseline construction produces a large *negative area*. If this is added to the area above the baseline, the result is a severe underestimate of the peak area. To avoid such errors, the integrator looks for negative areas and redraws the baseline as shown in Figure 24.

Peak Separation Codes

Figure 24

Tangent Skimming Small Peaks on Solvent Tail

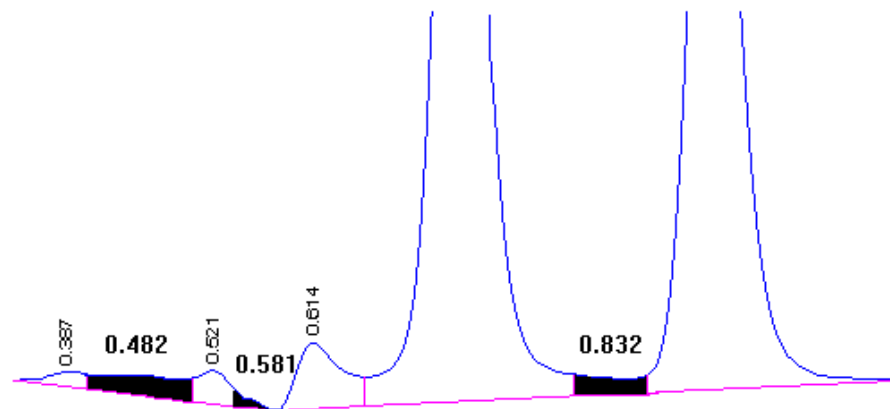


Unassigned Peaks

With some baseline constructions, there will be small areas that are above the baseline and below the signal, but are not part of any recognized peaks. Normally such areas are neither measured nor reported. If unassigned peaks is turned on, these areas are measured and reported as unassigned peaks. The *retention time* for such areas is the midpoint between the start and end of the area, as shown in Figure 25.

Figure 25

Unassigned Peaks



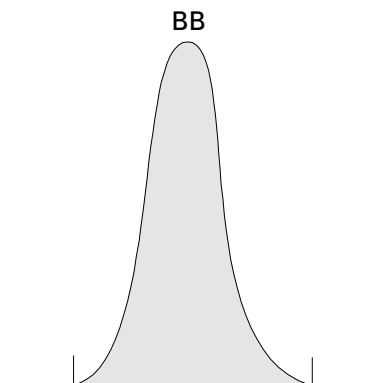
Peak Area Measurement

The last step in peak integration is determining the final area of the peak.

During the run, area under the baseline is accumulated and stored. Peak areas are calculated from the contents of the cardinal point file. Recall that cardinal points are the points chosen by the integrator to define and quantify a peak. These include baseline points, valley points, peak apex, points at peak half height. Cardinal points have a horizontal coordinate of elapsed time, a vertical coordinate of height from the baseline, area, and other parameters the integrator uses to calculate the peak areas.

Figure 26

Area Measurement for Baseline-to-Baseline Peaks



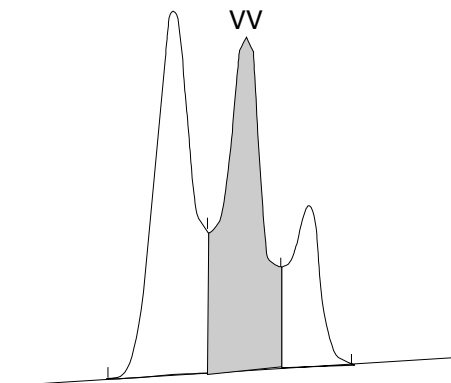
In the case of a simple, isolated peak, the peak area is determined by the accumulated area above the baseline between peak start and stop (identified by tick marks).

The area which the integrator calculates during integration is determined as follows:

- for baseline-to-baseline (BB) peaks, the area is above the baseline between the tick marks,
- for valley-to-valley (VV) peaks, the area above the baseline is segmented with vertical dropped lines from tick marks,

Figure 27

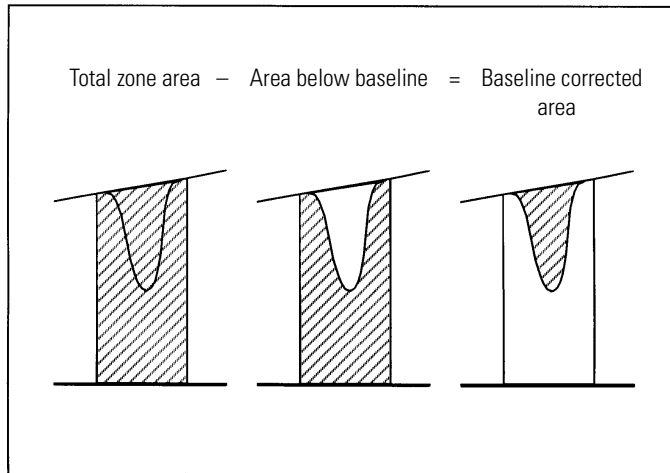
Area Measurement for Valley-to-Valley Peaks



- for tangent peaks, the area is above the reset baseline,
- for solvent peaks, the area is above the horizontal extension from the last-found baseline point and below the reset baseline given to tangent (T) peaks. A solvent peak may rise too slowly to be recognized, or there may be a group of peaks well into the run which you feel should be treated as a solvent with a set of riders. This usually involves a merged group of peaks where the first one is far larger than the rest. The simple dropline treatment would exaggerate the later peaks because they are actually sitting on the tail of the first one. By forcing the first peak to be recognized as a solvent, the rest of the group is skimmed off the tail.
- Negative peaks that occur below the baseline, have a positive area as shown in Figure 28.

Figure 28

Area Measurement for Negative Peaks



Symmetry

The enhanced integrator deals with peak symmetry in the following way:

- if peak width calculation is area/height, symmetry is calculated as front area/rear area (this calculation is also used when the front or rear inflection point is not detected, or
- otherwise, symmetry is calculated just as it is for the standard integrator.

Integration Events

The enhanced integrator provides the user with a number of initial and timed integrator events. Many events are on/off or start/stop pairs.

Initial Events

Initial Peak

Width

Sets the integrator's internal peak width to this value for the start of run. This initial peak width value is used to scale the accumulator that detects peak upslope, downslope, and tailing. The integrator updates it when necessary during the run to optimize the integration. It is entered by the user in units of time that correspond to the peak width at half-height of the first expected peak (excluding the solvent peak).

Slope

Sensitivity

Is the setting for peak sensitivity. In the enhanced integrator, the slope sensitivity setting changes on a linear scale. This is unlike the standard integrator which uses a height slope sensitivity expressed in powers of two.

Height reject

Height reject is a user parameter that sets the noise rejection for integration. Peaks smaller than this value will be ignored by the integrator. The higher the height reject setting, the greater the height above the established baseline must be before a data point can qualify as the start of a peak.

Height reject should not be used to reject peaks whose heights approach that of the smallest peaks of interest. Instead, use the Area Reject parameter to exclude small peaks.

Area reject

The area reject sets peak rejection by final area. Any peaks that have areas less than the minimum area will not be reported.

Shoulder detection

When shoulder detection is on, the integrator detects shoulders using the curvature of the peak as given by the

Integration Events

second derivative. When the curvature goes to zero, the integrator identifies this point of inflection as a possible shoulder. If the integrator identifies another point of inflection before the apex of the peak, a shoulder has been detected.

Peak Width

The calculation algorithm for the peak width has not changed with respect to the standard integrator. Please refer to the appropriate section on the standard integrator (see “Peak Width” on page 84).

The peak width setting controls the selectivity of the integrator to distinguish peaks from baseline noise. To obtain good performance, the peak width must be set close to the width at half-height of the actual chromatographic peaks. The integrator updates the peak width when necessary during the run to optimize the integration.

Choosing Peak Width

Choose the setting that provides just enough filtering to prevent noise being interpreted as peaks without distorting the information in the signal.

- To choose a suitable initial peak width for a single peak of interest, use the peak’s time width as the base as a reference.
- To choose a suitable initial peak width when there are multiple peaks of interest, set the initial peak width to a value equal to or less than the narrowest peak width to obtain optimal peak selectivity.

If the selected initial peak width is too low, noise may be interpreted as peaks. If broad and narrow peaks are mixed, you may decide to use runtime programmed events to adjust the peak width for certain peaks. Sometimes, peaks become significantly broader as the analysis progresses. Isothermal GC and Isocratic LC analyses have this characteristic. To compensate for this, the integrator automatically updates the Peak Width as peaks broaden during an analysis automatically unless disabled or set with a timed event.

The Peak Width update is weighted in the following way:

$$[0.75 \times (\text{existing peak width}) + 0.25 \times (\text{width of current peak})]$$

If a timed integration event disables or sets the peak width to a specific value, the automatic peak width adjustment is disabled.

Changing Height Reject and Peak Width

Both peak width and height reject are very important in the integration process. You can achieve different results by changing these values.

- Increase both the height reject and peak width where relatively dominant components must be detected and quantified in a high noise environment. An increased peak width improves the filtering of noise and an increased height reject ensures that random noise is ignored.
- Decrease height reject and peak width for detecting and quantifying trace components, those whose heights approach that of the noise level. Decreasing peak width decreases signal filtering, while decreasing height reject ensures that small peaks are not rejected because they have insufficient height.

When an analysis contains peaks with varying peak widths, peak width should be set for the more narrow peaks and the height reject should be reduced to ensure that the broad peaks are not ignored because of their reduced height.

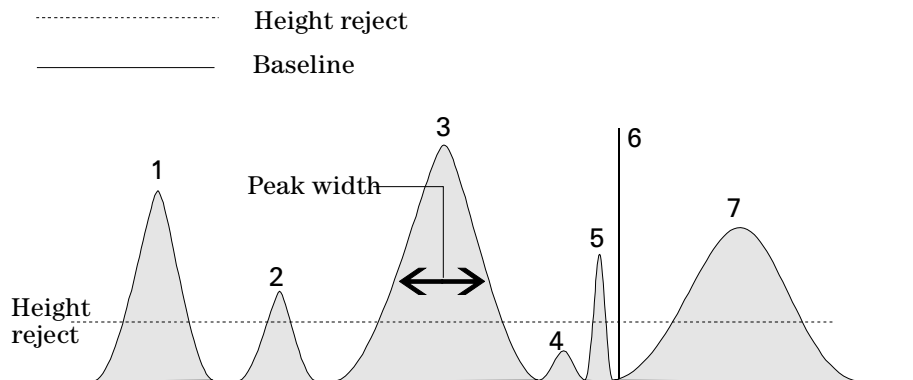
Customizing Integration

It is often useful to change the values for the slope sensitivity, peak width, height reject, and area reject to customize integration.

Figure 29 shows how these parameters affect the integration of five peaks in a signal.

Figure 29

Using Initial Events



Integration Events

A peak is only integrated when all of the three integration event requirements are satisfied. Using the peak width for peak 3, the area reject and slope sensitivity shown in Figure 29, only peaks 1 and 3 will be integrated.

Peak 1	is integrated as all three integration event requirements are satisfied.
Peak 2	is rejected because the area is below the set area reject value.
Peak 3	is integrated as all three integration event requirements are satisfied.
Peak 4	is not integrated because the peak height is below the Height Reject.
Peak 5	is integrated.
Peak 6	is not integrated because the peak is interpreted as a spike.
Peak 7	is integrated.

Table 7**Slope Sensitivity Values**

Integration Event	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
Height reject	Above	Above	Above	Below	Above	Above	Above
Area reject	Above	Below	Above	Below	Above	Above	Above
Minimum width reject	Above	Above	Above	Above	Above	Below	Above
Peak Integrated	Yes	No	Yes	No	Yes	No	Yes

Timed Events

You can use timed events to customize signal baseline construction when default construction is not appropriate. These events can be useful for summing final peak areas and for correcting short and long term baseline aberrations. For further information about integration events see your online help.

Integration Events Tables

Each signal (chromatogram or electropherograph) acquired in a particular method may have its own set of integration parameters saved in the Integration Events Table.

A user can create an Integrator Events Table in the following ways:

- manually editing the integration events, or
- selecting Autointegrate to set the initial values for each signal.

When the individual signal events table is not used, all signals will use the default integration events table for the corresponding detector. For each detector type there is a default integration events table, for example, FID, ECD or DAD. The default integration events tables cannot be deleted and are always part of the data analysis method.

Using the Enhanced Integrator

The enhanced integrator for the ChemStation provides the following tools for performing integration on a data set:

- integration,
- autointegration, and
- manual integration.

Integrate

Integrates the signal according to the values in the integration events table. You can use this function to adjust the integration results by changing the integration events, and then reintegrating the signal.

Both Autointegrate and Integrate provide integration results. However, autointegration automatically calculates the initial events of slope sensitivity and peak width, whereas the Edit Integration Event function allows you to specify these integration events or use the default values.

AutoIntegrate

AutoIntegrate for the enhanced integrator has been modified to make the selected parameters more robust and to supply user feedback about problems that AutoIntegrate encounters.

AutoIntegrate examines the beginning and end regions of the chromatogram to estimate noise and assigns appropriate initial values of Slope Sensitivity and Height Reject. AutoIntegrate also determines a **temporary** value for PeakWidth based on the run time and minimum detection criteria. With the Area Reject set to zero, a trial integration is performed. If few peaks are detected in the trial integration, the parameters are adjusted and the trial is repeated, several times if necessary.

After a suitable trial integration, AutoIntegrate uses widths from the earliest peaks found to compute an initial PeakWidth. Peak symmetry is used to exclude poorly shaped peaks from the calculation.

AutoIntegrate searches between the detected peaks for a confirmed segment of baseline. If found, AutoIntegrate uses this along with PeakWidth data to **refine** the values for Slope Sensitivity and Height Reject. A value of Area Reject is computed based on the initial Peakwidth and noise level.

AutoIntegrate issues warnings in the message line when:

- Peak symmetry requirements for the Peak Width calculations are relaxed
- Insufficient peaks are found for accurate Peakwidth calculations
- No peaks are found (AutoIntegrate tries to provide a 10:1 signal-to-noise ratio, even if this results in no peaks being detected)

Limitations

AutoIntegrate is a useful starting point for most chromatograms and in the early stages of method development. AutoIntegrate should not, however, be used or accepted as a substitute for good sample preparation and chromatographic techniques.

AutoIntegrate determines values for the **initial time** of the run which limits AutoIntegrate's ability to deal with unusual situations that may occur **later** in the chromatogram. AutoIntegrate may have problems dealing with

AutoIntegrate

chromatograms having disturbances or peaks at the start or the end of the run.

AutoIntegrate will have difficulty if the chromatogram requires integration timed events to deal with disturbances such as:

- Inverted peaks
- Baseline upsets and transients (valve or signal switched)
- Rising baseline at the end of the run (column bleed)

In the chromatogram above, the following integration timed events are used.

Integrator Off

Integrator On, Baseline All Valleys

Set Baseline

With the required timed events in place, AutoIntegrate can provide good performance.

Manual Integration

This type of integration allows you to integrate selected peaks or groups of peaks. Except for the initial area reject value, the ChemStation's event integration is ignored within the specified range of manual integration.

Manual integration enables you to define the peak start and stop points, and then include the recalculated areas in the quantification and reporting packages. Manually integrated peaks in the reports are annotated using the peak separation code M.

Manual Integration offers the following features:

Draw

Baseline Specifies where the baselines are to be drawn for a peak or set of peaks. You can also specify whether peaks in the range given should be automatically separated at all valley points.

Negative Peaks Specifies when to treat any areas below the baseline as negative peaks. You can also specify whether peaks in the range given should be automatically separated at all valley points.

Tangent

Skim Calculates the areas of peaks tangentially skimmed off a main peak. You can also specify whether peaks in the range given should be automatically separated at all valley points. The area of the tangent skimmed peak is subtracted from the area of the main peak.

Split

Peak Specifies a point where to split a peak with a dropline.

All

Valleys All Valleys specifies that the peaks within the chosen range of integration will be separated by constructing the baseline from the points at each valley.

Delete

Peak(s) Deletes one or more peaks from the integration results.

Peak Separation Codes

- Manually integrated peaks are reported in the integration reports by the peak code MM.
- If there is a peak before the manually integrated peak and the end of this peak changes because of the manual integration, it will have a code F (for forced).
- A solvent peak which has been affected by manual integration, such as tangent skim, will be labeled with an R (for re-calculated solvent) instead of an S.

Manual Integration Process

- The first step of manual integration is to select an area of the signal to integrate manually. This is done by zooming into the area of interest.
- The next step is to choose Draw Baselines, Negative Peaks, Tangent Skim or Delete Peak(s).
- The baselines can now be drawn by dragging the mouse while holding down the left mouse button. As the mouse is moved, a line is drawn from the starting point to the point where the mouse is currently positioned. In the case of Delete Peak(s) a rectangle surrounding the peaks to be deleted is drawn.
- The graphical input operates in two modes Absolute (default) and Trace. In either mode the message line displays the time and level of the cursor.
- In the Trace mode, the cursor is a down-pointing arrow and follows the shape of the signal, moving from data point to data point. You can activate the trace mode when the cursor is positioned at the desired start of a peak, by pressing the right button of your mouse.
- In Absolute mode, the cursor does not follow the signal but allows you to position the cursor any place on the screen. You can move from one mode to the other by clicking the right mouse button.
- For each peak manually integrated, the area counts will be displayed with the retention/migration time label on the screen.

Copying Manual Events to the Method

The software allows you to copy the integration events you defined using manual integration to the currently loaded method using the Copy Manual

Manual Integration

Events to Method item from the Integration menu. These manual events are then stored separately in the method. They cannot be reviewed in the Integration Events dialog box. When a signal is loaded they are only applied, when the Apply Manual Integration Events option is checked in the Integration Events dialog box or when you select Apply Manual Events from Method in the Integration Menu.

The manual integration events use absolute time values. They do not adjust for signal drift.

Peak Identification

Peak Identification

This chapter describes the following:

- what is peak identification?
- peak matching rules,
- types of peak identification,
- absolute retention time,
- relative retention time,
- peak qualifiers, and
- the identification process.

What is Peak Identification?

Peak identification identifies the components in an unknown sample based on their chromatographic/electropherographic characteristics determined by the analysis of a well-defined calibration sample.

The identification of these components is a necessary step in quantification if the analytical method requires quantification. The signal characteristics of each component of interest are stored in the calibration table of the method.

The function of the peak identification process is to compare each peak in the signal with the peaks stored in the calibration table.

The calibration table contains the expected retention/migration times of components of interest. A peak that matches the retention/migration time of a peak in the calibration table is given the attributes of that component, for example, the name and response factor. Peaks that do not match any of the peaks in the calibration table are classified as unknown. The process is controlled by:

- the retention/migration time in the calibration table for peaks designated as time reference peaks,
- the retention/migration time windows specified for reference peaks,
- the retention/migration times in the calibration table for the calibrated peaks that are not time reference peaks,
- the retention/migration time window specified for these non-reference peaks, and
- the presence of any additional qualifying peaks in the correct ratios.

Peak Matching Rules

The following rules apply to the peak matching process:

- if a sample peak falls within the peak matching window of a component peak from the calibration table, the peak is given the attributes of that component,
- if more than one sample peak falls within the peak matching window, then, the peak closest to the expected retention/migration time is identified as that component,
- if a peak is a time reference or internal standard, then the largest peak in the window is identified as that component,
- if peak qualifiers are also used then the peak ratio is used in combination with the peak matching window to identify the component peak,
- if the peak is a qualifier peak, the measured peak closest to the main peak of the compound is identified, and
- if a sample peak does not fall in any peak matching window, it is listed as an unknown component.

Types of Peak Identification

There are different techniques that can be used to match sample peaks with those in the calibration table of the ChemStation software.

Absolute Retention/Migration Time

The retention/migration time of the sample peak is compared with the expected retention/migration time specified for each component in the calibration table.

Corrected Retention/Migration Time

The expected retention/migration times of component peaks are corrected using the actual retention/migration times of one or more reference peaks, and the matching process is done using these corrected (relative) retention/migration times. The reference peak or peaks must be specified in the calibration table.

Peak Qualifiers

In addition to identifying peaks by retention/migration time, you can use peaks qualifiers to allow a more precise result. If more than one peak occurs in a retention/migration time window then qualifiers should be used to identify the correct compound.

Amount Limits

The amount limits defined in the Compound Details dialog box are used to qualify the peak identification. If the amount of the identified compound is inside the amount limits the peak identification is indicated in the report.

Absolute Retention/Migration Time

A retention/migration time window is used in the peak matching process. The retention/migration time window is a window which is centered on the retention/migration time for an expected peak. Any sample peak that falls within this window may be considered as a candidate for component identification.

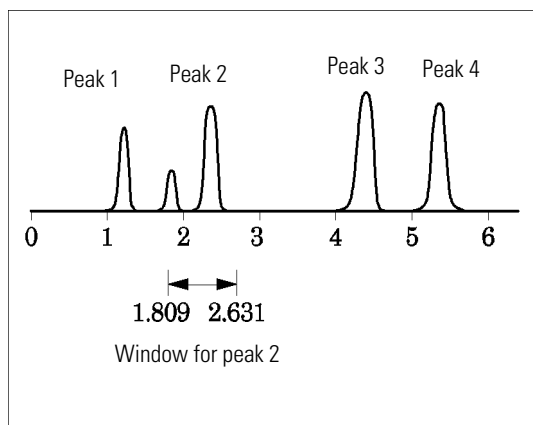
Figure 30 shows a retention/migration time window for peak 2 which is between 1.809 and 2.631 minutes where the expected retention/migration time is 2.22 minutes. There are two possibilities for peak 2. One is at 1.85 minutes and the other at 2.33 minutes. If the expected peak is a non-reference peak, the peak closest to the expected retention/migration time of 2.22 minutes is selected.

If the expected peak is a time reference or internal standard, the largest peak in the window is selected.

In both cases the ChemStation selects the peak at 2.33 minutes. If the two peaks were the same size then the peak closest to the center of the window is chosen.

Figure 30

Retention/Migration Time Windows



Three types of windows are used when trying to locate peaks.

- reference peak windows which apply to reference peaks only,

Absolute Retention/Migration Time

- non-reference peak windows which apply to all other calibrated peaks, and
- specific window values for individual components which are set in the Compound Details dialog box.

The default values for these windows are entered in the Calibration Settings dialog box. The width on either side of the retention/migration time that defines the peak matching window is the sum of the absolute and percentage windows.

A window of 5 % means the peak must have a retention/migration time between less than 2.5 % and more than 2.5 % of the calibrated retention/migration time for that peak. For example, a peak with a retention/migration time of 2.00 in the calibration run must appear between 1.95 and 2.05 minutes in subsequent runs.

For example, an absolute window of 0.20 minutes and a relative window of 10 % gives a retention/migration time window of between 1.80 and 2.20 minutes.

$$1.80 \text{ min} = 2.00 \text{ min} - 0.10 \text{ min} (0.20 \text{ min} / 2) - 0.10 \text{ min} (10 \% \text{ of } 2.00 \text{ min}).$$

$$2.20 \text{ min} = 2.00 \text{ min} + 0.10 \text{ min} (0.20 \text{ min} / 2) + 0.10 \text{ min} (10 \% \text{ of } 2.00 \text{ min}).$$

Corrected Retention/Migration Times

To match peaks by absolute retention/migration times may be simple but not always reliable. Individual retention/migration times may vary slightly due to a small change in conditions or technique. As a result peaks may occur outside the peak matching windows and therefore are not identified.

A technique to deal with the inevitable fluctuations that occur in absolute retention/migration times is to express component retention/migration times relative to one or more reference peaks.

Reference peaks are identified in the calibration table with an entry in the reference column for that peak. The relative peak matching technique uses the reference peak or peaks to modify the location of the peak matching windows in order to compensate for shifts in the retention/migration times of sample peaks.

If no reference peak is defined in the method or the ChemStation cannot identify at least one reference peak during the run, the software will use absolute retention/migration times for identification.

Single Reference Peaks

A retention/migration time window for the reference peak is created around its retention/migration time. The largest peak falling within this window is identified as the reference peak. The expected retention/migration times of all other peaks in the calibration table are corrected, in proportion to the ratio of the expected retention/migration time to the actual retention/migration time of the reference peak.

Multiple Reference Peaks

Correcting retention/migration times with a single reference peak is based on the assumption that the deviation of actual retention/migration time from the expected retention/migration times changes uniformly and linearly as the run progresses. Often during a long run the retention/migration time changes non-uniformly. In such cases better results are obtained using multiple reference peaks spaced at intervals across the run. This splits the signal into separate zones. Within each zone the deviation between retention/migration times is assumed to change linearly, but the rate of change is determined separately for each zone.

NOTE

The time correction algorithm may fail if the retention times of multiple reference peaks are too close to each other and are not distributed across the total run time.

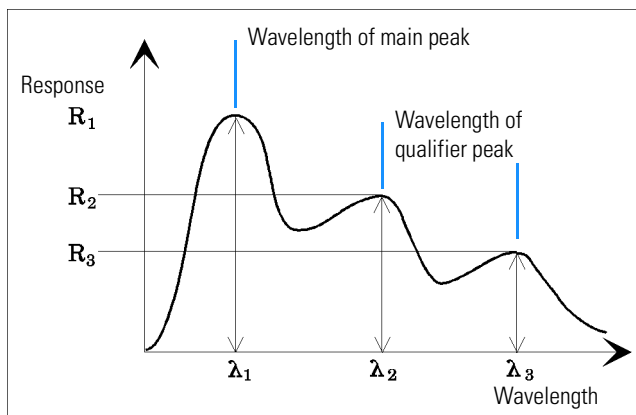
Peak Qualifiers

A component can be detected with more than one signal. Although applicable to all forms of chromatography using multiple detectors or detectors capable of producing multiple signals, multisignal detection is most commonly used in liquid chromatography with multiple wavelength or diode array detectors. Such detectors are normally set up so that the wavelength closest to the greatest absorbance (area) is used to define the main peak in the calibration table. In Figure 31 this is λ_1 .

The two other wavelengths that were acquired as signals can be used as peak qualifiers. In Figure 31 these are λ_2 and λ_3 .

Figure 31

Peak Qualifiers



Peaks of a compound have a constant response ratio over different wavelengths.

The qualifier peak response is a certain percentage of the main peak response. Limits which determine the acceptable range for the expected response can be set in the calibration table when the Identification Details option is selected. If the ratio between the main peak qualifier λ_1 and the qualifier peak, for example, λ_3 is within the allowed limits then the compound identity can be confirmed.

Signal Correlation

Signal correlation means that two peaks measured in different detector signals within a defined time window are assigned to the same compound. The signal correlation window can be controlled by the SignalCorrWin parameter in the QuantParm table of the _DaMethod register. Signal correlation is disabled when setting the signal correlation window to 0.0 minutes (see the *Macro Programming Guide* for more information). When signal correlation is off, peaks eluting at the same retention time in different detector signals are treated as different compounds.

The default signal correlation window for LC, LC/MS and CE data is 0.03 minutes and 0.0 minutes for GC data.

Qualifier Verification

If signal correlation is enabled, qualifier verification is active for all data file types by default. It can be disabled by setting the UseQualifiers flag in the Quantification Parameters table of the method (see the macro programming guide for more details). Qualifier verification is also disabled when signal correlation is switched off.

Qualifier Ratio Calculation

When qualifiers verification is enabled for a compound, the ratio of the qualifier size and the main peak size is verified against the calibrated limits. The size may be height or area according to the calculation base setting in Specify Report.

The qualifier peaks can be calibrated in the same way as the target compounds. The user does not need to specify the expected qualifier ratio. The expected qualifier ratio is calculated automatically:

$$\text{QualRespRatio} = \text{Response of the qualifier} / \text{Response of the main peak}$$
both measured at the retention time of the compound.

The QualTolerance parameter defines the acceptable range of the qualifier ratio, for example, $\pm 20\%$.

The tolerance can be set in the calibration table user interface (Identification Details) and is an absolute percentage. For example, absolute percentage:

Acceptable range = $50\% \pm 20\% = 30\% \dots 70\%$

The Identification Process

When attempting to identify peaks, the software makes three passes through the integration data.

Finding the Reference Peaks

The first pass identifies the time reference peaks. The software searches peak retention/migration times from a run for matches within the retention/migration windows of the reference peaks in the calibration table. A peak from the run is identified as a reference peak in the calibration table if the run peak's retention/migration time is within the window constructed for the calibration table peak.

If more than one peak is found within a window, the peak with the largest area or height followed by a positive signal qualifier match, if set up, is chosen as the reference peak.

After each time reference peak is found, the difference between its retention/migration time and that given in the calibration table is used to adjust the expected retention/migration times of all other peaks in the Calibration table.

Finding the ISTD Peaks

The second pass identifies any defined internal standard peaks. If they have not already been identified as ISTD, peaks may be identified as time reference peaks. ISTD peaks are identified by peak retention/migration time windows and peak qualifiers. If more than one peak is found in the same ISTD window, the largest peak is chosen.

Finding the Remaining Calibrated Peaks

The third pass identifies all remaining peaks listed in the calibration table. The non-reference peaks in the calibration table are matched to the remaining run peaks by using their RT window.

Each non-reference calibrated peak has its own retention/migration time in the calibration table. This is adjusted for the particular run based on the pre-identification of the time reference peaks. The retention/migration time

The Identification Process

window of the calibrated peak is adjusted based on the corrected retention/migration time of the calibrated peak.

If more than one peak is found in the same window, the peak with a retention/migration time which is closest to the expected retention/migration time and also meets the optional qualifier specifications is chosen.

Classification of Unidentified Peaks

If there are remaining peaks, which are still not identified, they are classified as unknown. The ChemStation attempts to group the unknown peaks that belong to the same compound. If a peak has been detected in more than one signal, the peaks with the same retention/migration time in each signal are grouped to one compound.

Unknown peaks are reported if the corresponding selection has been made in the Calibration Settings dialog box.

Peak Identification
The Identification Process

Quantification

Quantification

This chapter describes the following:

- what is quantification?
- quantification calculations,
- area% and height% calculations,
- external standard (ESTD) calculation,
- norm% calculation,
- internal standard (ISTD) calculation, and
- quantification of unidentified peaks.

What is Quantification?

After the peaks have been integrated and identified, the next step in the analysis is quantification. Quantification uses peak area or height to determine the concentration of a compound in a sample.

A quantitative analysis involves many steps which are briefly summarized as follows:

- Know the compound you are analyzing.
- Establish a method for analyzing samples containing this compound.
- Analyze a sample or samples containing a known concentration or concentrations of the compound to obtain the response due to that concentration.

You may alternatively analyze a number of these samples with different concentrations of the compounds of interest if your detector has a non-linear response. This process is referred to as **multi-level calibration**.

- Analyze the sample containing an unknown concentration of the compound to obtain the response due to the unknown concentration.
- Compare the response of the unknown concentration to the response of the known concentration to determine how much of the compound is present.

To obtain a valid comparison for the unknown sample response to that of the known sample, the data must be acquired and processed under identical conditions.

Quantification Calculations

The ChemStation offers the following calculation procedures for determining the concentration of each component present in a mixture:

- Percent
- Normalization
- External standard (ESTD)
- ESTD%
- Internal standard (ISTD)
- ISTD%

The calculations used to determine the concentration of a compound in an unknown sample depend on the type of quantification. Each calculation procedure uses the peak area or height for the calculation and produces a different type of report.

Correction Factors

The quantification calculations use four correction factors, the **absolute response factor**, the **multiplier**, the **dilution factor**, and the **sample amount**. These factors are used in the calibration procedures to compensate for variations in detector response to different sample components, concentrations, sample dilutions, sample amounts, and for converting units.

Absolute Response Factor

The absolute response factor for a sample component represents the amount of the component divided by the measured area or height of the component's peak in the analysis of a calibration mixture. The absolute response factor, which is used by each calibrated calculation procedure, corrects for detector response to individual sample components.

Multiplier

The multiplier is used in each calculation formula to multiply the result for each component. The multiplier may be used to convert units to express amounts.

Dilution Factor

The dilution factor is a number by which all calculated results are multiplied before the report is printed. You can use the dilution factor to change the scale of the results or correct for changes in sample composition during pre-analysis work. You can also use the dilution factor for any other purposes that require the use of a constant factor.

Sample Amount

If the ESTD% or ISTD% calculations are selected, the ESTD and ISTD reports give relative values rather than absolute values, that is, the amount of each component is expressed as a percentage of the sample amount. The sample amount is used in ESTD% and ISTD% reports to convert the absolute amount of the components analyzed to relative values by dividing by the value specified.

Uncalibrated Calculation Procedures

Uncalibrated calculation procedures do not require a calibration table.

Area% and Height%

The Area% calculation procedure reports the area of each peak in the run as a percentage of the total area of all peaks in the run. Area% does not require prior calibration and does not depend upon the amount of sample injected within the limits of the detector. No response factors are used. If all components respond equally in the detector and are eluted, then Area% provides a suitable approximation of the relative amounts of components.

Area% is used routinely where qualitative results are of interest and to produce information to create the calibration table required for other calibration procedures.

The Height% calculation procedure reports the height of each peak in the run as a percentage of the total height of all peaks in the run.

Calibrated Calculation Procedures

The external standard (ESTD), normalization, and internal standard (ISTD) calculation procedures require response factors and therefore use a calibration table. The calibration table specifies conversion of responses into the units you choose by the procedure you select.

ESTD Calculation

The ESTD procedure is the basic quantification procedure in which both calibration and unknown samples are analyzed under the same conditions. The results from the unknown sample are then compared with those of the calibration sample to calculate the amount in the unknown.

The ESTD procedure uses absolute response factors unlike the ISTD procedure. The response factors are obtained from a calibration and then stored. In following sample runs, component amounts are calculated by applying these response factors to the measured sample amounts. One precaution that must be observed in this type of calculation is that the sample injection size must be reproducible from run to run, since there is no standard in the sample to correct for variations in injection size or sample preparation.

When preparing an ESTD report, the calculation of the amount of a particular compound in an unknown sample occurs in two steps:

- 1 An equation for the curve through the calibration points for this compound is calculated using the type of fit specified in the Calibration Settings or Calibration Curve dialog box.
- 2 The amount of the compound in the unknown is calculated using the equation described below. This amount may appear in the report or it may be used in additional calculations called for by Multiplier, Dilution Factor, or Sample Amount values before being reported.

If the ESTD report is selected, the equation used to compute the absolute amount of component x is:

$$\text{Absolute Amt of } x = \text{Response}_x \cdot \text{RF}_x \cdot M \cdot D$$

where:

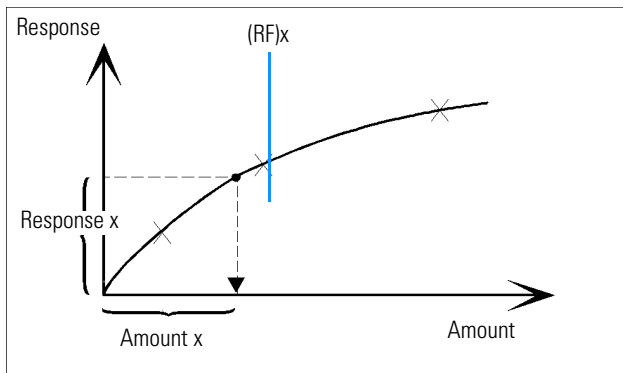
Response_x is the response of peak x ;

RF_x is the response factor for component x , calculated as: $\text{RF}_x = \frac{\text{Amount}_x}{\text{Response}_x}$

M is the multiplier.

Quantification
ESTD Calculation

D is the dilution factor.



The multiplier and dilution factor are read either from the Calibration Settings or from the Sample Information dialog box.

If the ESTD% report is selected and sample amount is not zero, the relative amount (%) of a component x is calculated as shown below:

$$\text{Relative Amt of } x = \frac{(\text{Absolute Amt of } x) \cdot 100}{\text{Sample Amount}}$$

where:

Absolute amount of x is calculated as shown above in the ESTD calculation;

Sample amount is obtained from the Sample Information box, or from the Calibration Settings dialog box for single runs. If sample amount is zero, the ESTD is calculated.

Norm% Calculation

In the normalization method, response factors are applied to the peak areas (or heights) to compensate for changes that occur in detector sensitivity for the different sample components.

The Norm% report is calculated in the same way as an ESTD report except that there is an additional step to calculate the relative rather than absolute amounts of compounds.

The Norm% report has the same disadvantage as the Area% and Height% reports. Any changes that affect the total peak area will affect the concentration calculation of each individual peak. The normalization report should only be used if all components of interest are eluted/migrated and integrated. Excluding selected peaks from a normalization report will change the reported results in the sample.

The equation used to calculate the Norm% of a component x is:

$$\text{Norm\% of } x = \frac{\text{Response}_x \cdot \text{RF}_x \cdot 100 \cdot M \cdot D}{\sum (\text{Response} \cdot \text{RF})}$$

where:

Response_x is the area (or height) of peak x;

RF_x is the response factor;

$\sum (\text{Response} \cdot \text{RF})$ is the total of all the (*RESPONSE*)(*RF*) products for all peaks including peak x;

M is the multiplier

D is the dilution factor.

The multiplier and dilution factor are read either from the Calibration Settings, or from the Sample Information dialog box.

ISTD Calculation

The ISTD procedure eliminates the disadvantages of the ESTD method by adding a known amount of a component which serves as a normalizing factor. This component, the **internal standard**, is added to both calibration and unknown samples.

The software takes the appropriate response factors obtained from a previous calibration stored in the method. Using the internal standard concentration and peak areas or heights from the run, the software calculates component concentrations.

The compound used as an internal standard should be similar to the calibrated compound, both chemically and in retention/migration time, but it must be chromatographically or electropherographically distinguishable.

Table 8

ISTD Procedure

Advantages

Sample-size variation is not critical.

Instrument drift is compensated by the internal standard.

The effects of sample preparations are minimized if the chemical behavior of the ISTD and unknown are similar.

Disadvantages

The internal standard must be added to every sample.

If the ISTD procedure is used for calibrations with a non-linear characteristic, care must be taken that errors which result from the calculation principle do not cause systematic errors. In multi-level calibrations, the amount of the ISTD compound should be kept constant, i.e. the same for all levels if the calibration curve of the compound is non-linear.

In the internal standard analysis, the amount of the component of interest is related to the amount of the internal standard component by the ratio of the responses of the two peaks.

In a two-run ISTD calibration, the calculation of the corrected amount ratio of a particular compound in an unknown sample occurs in the following stages:

Run 1: Calibration

- 1 The calibration points are constructed by calculating an amount ratio and a response ratio for each level of a particular peak in the calibration table.

The amount ratio is the amount of the compound divided by the amount of the internal standard at this level.

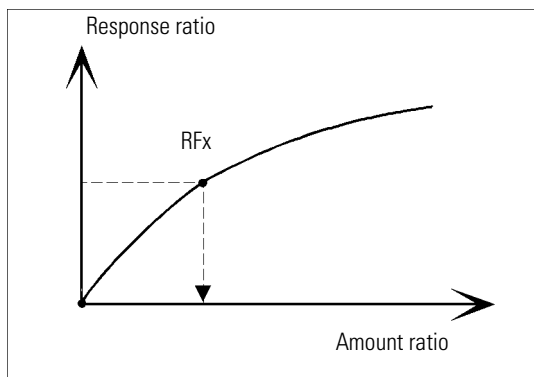
The response ratio is the area of the compound divided by the area or height of the internal standard at this level.

- 2 An equation for the curve through the calibration points is calculated using the type of curve fit specified in the Calibration Settings dialog box or Calibration Curve dialog box.

$$RF_x = \frac{\text{Amount Ratio}}{\text{Response Ratio}}$$

Figure 32

Amount Ratio



Run 2: Unknown Sample

- 1 The response of the compound in the unknown sample is divided by the response of the internal standard in the unknown sample to give a response ratio for the unknown.
- 2 An amount ratio for the unknown is calculated using the curve fit equation determined in step 2 above, and the actual amount of ISTD in the sample.

ISTD Calculation of Calibrated Peaks

The equations used to calculate the actual amount of a calibrated component x for a single-level calibration are:

$$\text{Response Ratio} = \frac{\text{Response}_x}{\text{Response}_{\text{ISTD}}}$$

$$\text{Actual Amt of } x = (\text{Response Ratio} \cdot \text{RF}_x) \cdot (\text{Actual Amount of ISTD}) \cdot M \cdot D$$

where:

RF_x is the response factor for compound x ;

The actual amount (*Actual Amt*) of ISTD is the value that was entered in the Calibration Settings dialog box or the Sample Info dialog box for the internal standard added to the unknown sample;

M is the multiplier.

D is the dilution factor.

If the ISTD% report type is selected, the following equation is used to calculate the relative (%) amount of component x :

$$\text{Relative Amt of } x = \frac{(\text{Actual Amount of } x) \cdot 100}{\text{Sample Amount}}$$

ISTD Calculation of Uncalibrated Peaks

There are two ways to define the response factor which is used to calculate the amount for unidentified peaks.

- 1 Use the fixed response factor set in the With Rsp Factor box of the Calibration Settings dialog box. You can choose to correct the fixed response factor by specifying an ISTD correction.

$$\text{Actual Amount of } x = \text{RF}_x \cdot (\text{Response Ratio})_x \cdot \text{Actual Amount of ISTD} \cdot M \cdot D$$

$$\text{Response Ratio} = \frac{\text{Response}_x}{\text{Response}_{\text{ISTD}}}$$

RF_x is the Response Factor set in the Calibration Settings dialog box.

You can see from these formulae that the variations in the ISTD response are used to correct the quantification of the unknown component.

- 2 Use a calibrated peak. This ensures that the same response factor is used for the quantification of all peaks. The response factor of the selected

ISTD Calculation

compound and the uncalibrated peaks is corrected during all recalibrations. If the calibrated peak response factor changes, then the response factor for the unidentified peaks also changes by the same amount. If a Calibration Table is already set up, you can select a compound from the Using Compound combo box in the Calibration Settings dialog box.

The equations used to calculate the actual amount of an uncalibrated peak x are shown in above.

Calibration

Calibration

This chapter describes the following:

- definitions of terms,
- calibration table,
- calibration curve,
- unknown samples,
- types of calibration,
- group calibration, and
- recalibration.

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. See Chapter 7 “Peak 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.

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, see Chapter 7 “Peak Identification”. 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.

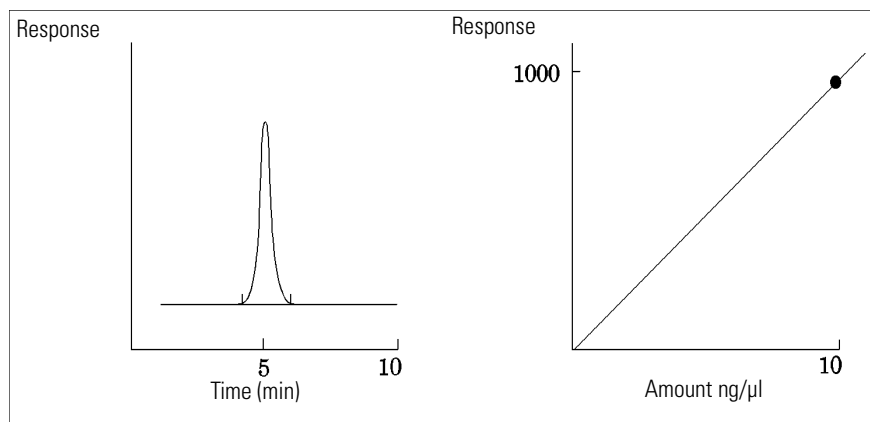
Calibration Curve

A calibration curve is a graphical presentation of the amount and response data for one compound obtained from one or more calibration samples.

Normally an aliquot of the calibration sample is injected, a signal is obtained, and the response is determined by calculating the area or height of the peak, similar to Figure 33.

Figure 33

Calibration Sample (10 ng/μl) Signal and Calibration Curve



A *correlation coefficient* is displayed with the graphic of the calibration curve. The correlation coefficient is the square root of the regression coefficient and gives a measure of the fit of the calibration curve between the data points. The value of the coefficient is given to three decimal places, in the range:

0.000 to 1.000

where:

0.000 = no fit

1.000 = perfect fit

Calibration Curve

For each calibration level the *relative residual* is displayed. It is calculated using the following formula:

$$\text{relRES} = \frac{\text{Response}_{\text{calibrated}} - \text{Response}_{\text{calculated}}}{\text{Response}_{\text{calculated}}} \cdot 100$$

where:

relRES= relative residual in percent

The calculated response represents the point on the calibration curve.

The *residual standard deviation*, which is printed on some reports and when selecting Print calibration table and curves is calculated using the following formula:

$$\text{ResSTD} = \sqrt{\frac{\sum_{i=1}^n (\text{Resp}_{\text{calibrated}i} - \text{Resp}_{\text{calculated}i})^2}{n - 2}}$$

where:

ResSTD = residual standard deviation

Resp_{calibratedi} = calibrated response for point i

Resp_{calculatedi} = calculated response for point i

n= number of calibration points

Unknown Samples

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

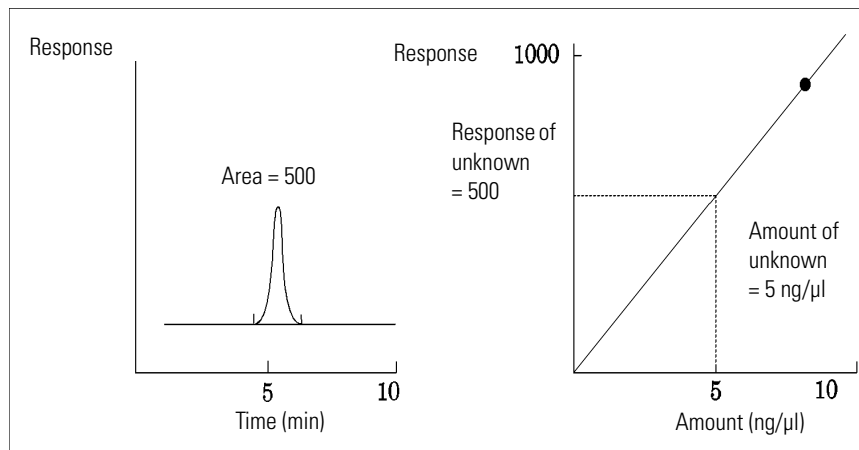
To find out how much of the compound is in the unknown sample, you must:

- 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/ μ l, by using the calibration curve shown in Figure 34.

Figure 34

Signal from Unknown Sample and Calibration Curve



Types of Calibration

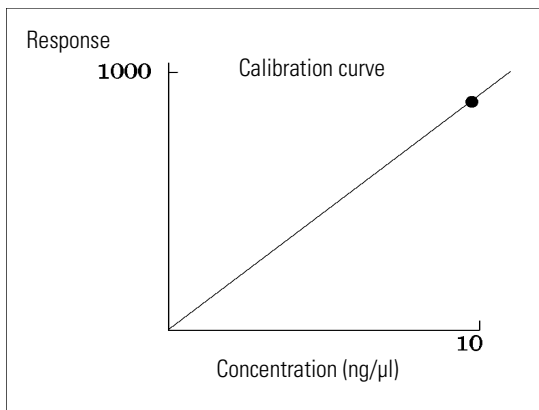
The ChemStation offers two types of calibrations, single-level and multilevel calibrations.

Single-Level Calibration

The calibration curve shown in Figure 35 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

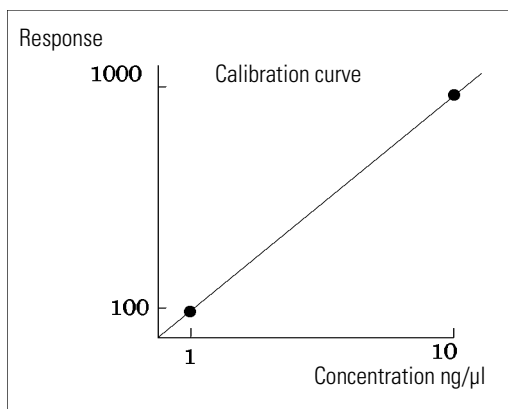
Single-Level Calibration Curve



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.

Amount Limits

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

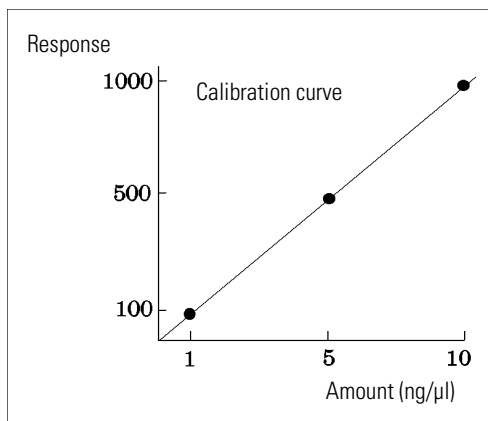
Multilevel Calibration

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

Multilevel 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 9.

Table 9

Calibration Table

Level	Amount (ng/ml)	Response (area counts)
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

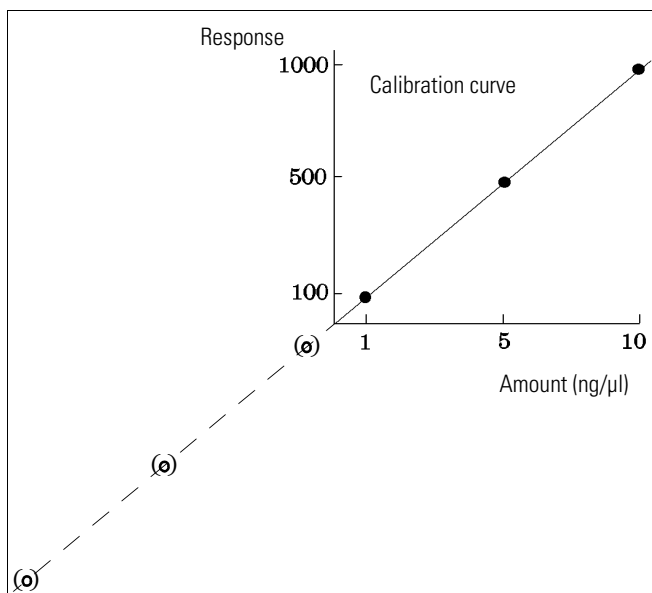
There are four ways to treat the origin when the response curve is plotted:

- 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 174.

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 $1/x \cdot a$ 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

Types of Calibration

$1/y$ 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 $(1/y) - b$ 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 $1/x^2 \cdot a^2$ 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 $1/y^2 \cdot b^2$ 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.

Group Calibration

Group calibration can be applied for compounds where the individual concentrations are not known but the sum of concentrations for a group of compounds is known. An example are isomers. Complete compound groups are calibrated. The following formulae are used:

Calibration

$$\text{Conc}_{AB} = \text{RF}_A \cdot \text{Response}_A + \text{RF}_B \cdot \text{Response}_B$$

where:

Conc_{AB} is the concentration of the compound group consisting of compound A and B

Response_A is the area (or height) of compound A

RF_A is the response factor

For compounds within a compound group we assume equal response factors:

$$\text{RF}_A = \text{RF}_B$$

Therefore the concentration of a compound within a compound group is calculated as follows:

$$\text{Conc}_A = \frac{\text{Conc}_{AB} \cdot \text{Resp}_A}{\text{Resp}_A + \text{Resp}_B}$$

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.

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/electropherography. 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.

Recalibration

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.

Recalibrations with Peak Summing

When a recalibration is performed, the retention 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.

Recalibration Options

You have several ways to update the responses in the calibration table with the new calibration data.

Average The average from all calibration runs are calculated using the following formula

$$\text{Response} = \frac{n \cdot \text{Response} + \text{MeasResponse}}{n + 1}$$

where

n = number of previous calibrations

MeasResponse = measured response

Floating Average

A weighted average for all calibration runs is calculated. The updated weight is set in the Recalibration Settings dialog box.

$$\text{Response} = \left(1 - \frac{\text{Weight}}{100}\right) \cdot \text{Response} + \left(\frac{\text{Weight}}{100}\right) \cdot \text{MeasResponse}$$

where

weight= response update weight set in Recalibration Settings

MeasResponse = measured response

Replace The new response values replace the old values.

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 196.

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

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.

Automation

Automation

This chapter describes the following:

- what is automation?
- what is a sequence?
- working with sequences,
- types of sequences,
- editing sequences,
- what happens when a sequence is run? and
- customizing 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 is a Sequence?

A sequence is a series of instructions that automates the analysis of samples.

A sequence 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, uses different sets of chromatographic or electropherographic conditions and evaluation parameters.

Sequence Parameters

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

- enter information about the data directory and operator name (the operator name entered in the access level dialog box is shown), and
- specify how the sequence processing should be done by choosing particular Part of Methods to Run parameters.

For example, you can choose to either:

- execute the run-time checklist,
- do acquisition only, or
- do reprocessing only.

If the reprocessing 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

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 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.

Working with Sequences

Sequences 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 using the Load Sequence item in the Sequence menu.

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 system.

Stopping a Sequence

The currently active run will be completed before the sequence stops. A stopped sequence can never be resumed.

Aborting a Sequence

The abort function terminates a currently active sequence immediately.

Pausing a Sequence

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 the ChemStation will process after the samples in the currently running sequence line.

Running a Partial Sequence

A sequence table that is already set up can be partially executed by selecting Partial Sequence from the Sequence menu. The system displays the Partial Sequence dialog box and allows you to select individual samples from the table for analysis.

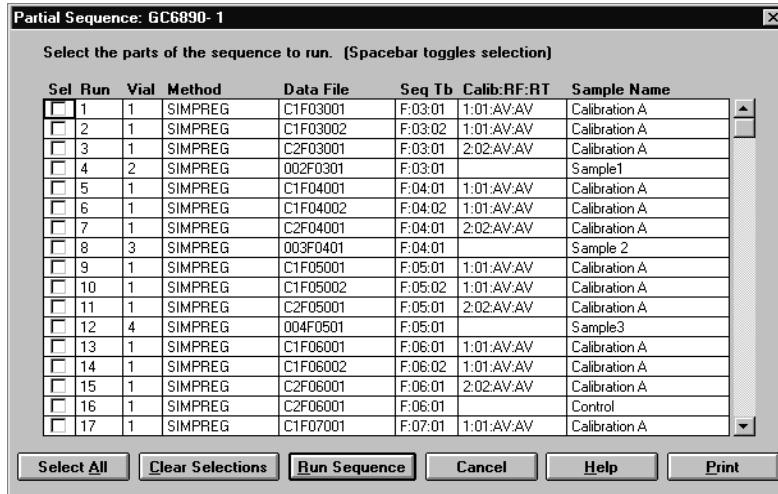
A single run is shown on each line of the Partial Sequence dialog box. For each run, the vial, method, data file, and sample name are given. Additionally, encoded information about the sequence table and any calibration samples are shown in the Seq Tbl and Calib:RF:RT columns. See the online help for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

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

The following Partial Sequence dialog box results when the SimpReg Method and Sequence Table, shown later in Table 14 and Table 15, are current. Samples 1, 2, 4, 5, and 8 are marked for processing.

Figure 39

Partial Sequence Dialog Box

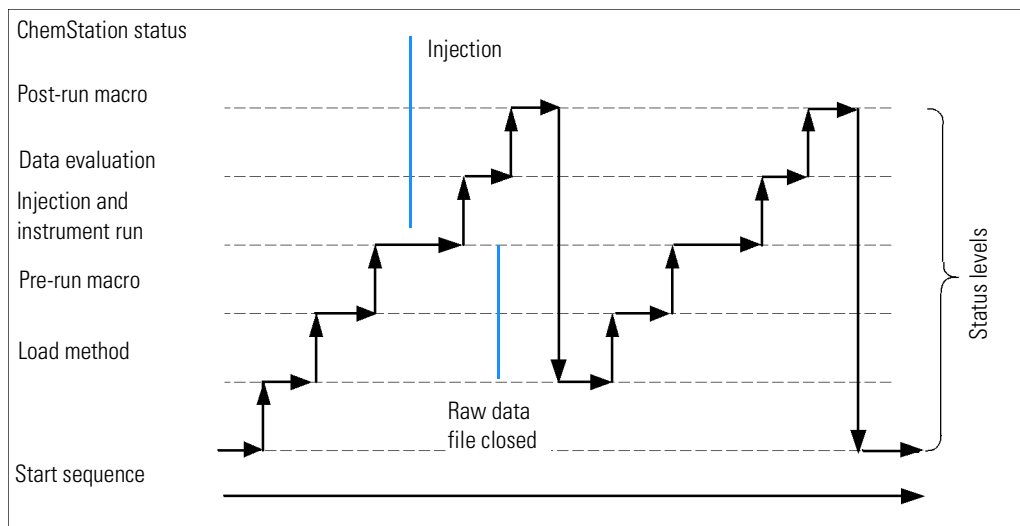


Sequence Log File

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?

- 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 method data evaluation is done. Integration, quantification, and reporting, including any user specified macro.
- The post-run macro is executed.
- During the entire process, the ChemStation tracks the sequence's progress in real time and produces a sequence log file.

Figure 40**Sequence Status**

Data File Naming in a Sequence

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

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

In each case, you should specify a subdirectory where the data files are to be stored, see “Sequence Parameters” on page 185.

Automatic Data File Naming During 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 (automatic or prefix-counter) is used to create the data file name. If any text is entered into the Datafile column, the 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, the 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, the ChemStation generates a name for each analysis. For an instrument that supports dual signal analysis such as the GC, the ChemStation will generate a name for each signal.

The ChemStation generates data file names from a user-supplied Prefix (1 to 7 alphanumeric characters) and a Counter (8 minus the number of characters in the prefix).

In the example below, TEST is the prefix and 0001 is the counter.

TEST0001

The counter is incremented each time a data file is generated.

Postsequence Operation

You can specify what happens after a sequence has finished during normal execution or when the 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 reequilibration 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.

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 Table 10.

Table 10

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%	

Table 10 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 11 **Sequence Table for Method A and Method B**

Line	Vial	Sample Name	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						...

Table 11 Sequence Table for Method A and Method B, continued

Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval	...
5	12		Method A	1						...
6	13		Method A	1						...
7	14		Method A	1						...
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

This section describes the analysis order for Method A which is the first part of the two-method sequence.

Method B Analysis Order

This section describes the analysis order for Method B, which is the second part of the two-method sequence.

Method B has the following differences compared with Method A:

- There are two injections per vial for calibration level 2. The Interval entry is set to 3.

Note that the results shown in Table 12 and Table 13 can be obtained by using Partial Sequence to see a preview of the run order after setting up the Sequence Table.

Table 12

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

Table 13

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

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 14 **Sequence Table for SIMPREG**

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.

Explicit and Cyclic Calibrations Together

- 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

This section describes the analysis order for the SimpReg method.

Table 15**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

This type of sequence interpolates calibrations over one or more unknown samples to more accurately represent the instrument response at the time the samples were analyzed. By using sequence bracketing, you can minimize the effects of instrument response drift on the calibration process.

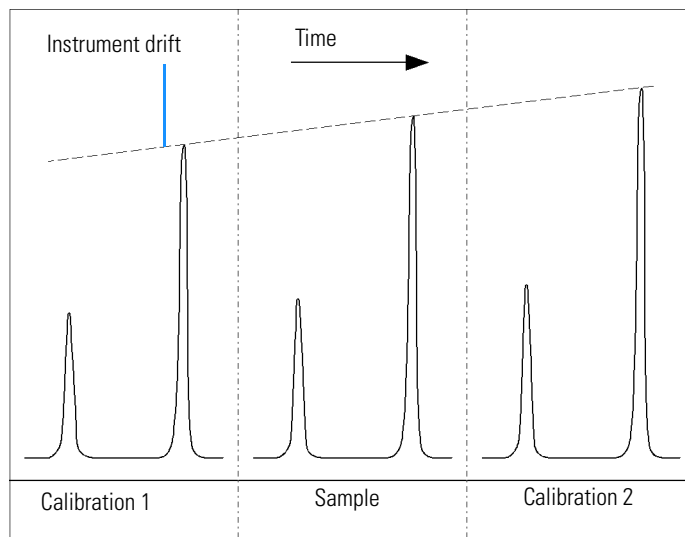
Example

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, see Figure 41.

To obtain a precise quantitative result for injection two (the sample), an interpolation must be made between the two calibration samples; see Figure 41. This process is known as bracketing.

Figure 41

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.

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 16

Sequence Table for BRACK-M

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						...

Bracketed Sequence Analysis Order

Run No.	Method Name	Vial No.	Inj No.	DataFile Name	Lvl No.	Upd RF	Upd Ret	Operation
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

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.

The execution order is:

- Vial 1 (Cal1a)
- Vial 5 (Cal2a)
- 6 injections out of vial 10 (Sample10)

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

Table 17 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				

- Vial 2 (Cal1b)
- Vial 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 Recalibration Sequences with Multiple Vials Containing the Same Dilution of a Standard

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

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

the recalibration interval has to be 1. The number of calibration lines per level is the number of samples plus one.

Table 18 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

Automation

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

Batch Review

Batch Review

This chapter describes the following:

- what is Batch Review?
- batch configuration
- review functions
- batch reporting

What is Batch Review?

Batch review is a capability within data analysis designed to help an analyst perform a “first-pass” review of the results of a sequence or a selection of runs quickly and easily. It will save time especially when reprocessing large numbers of samples. Whenever a sequence is run, a batch file (with a .b extension) is automatically generated and placed in the data directory along with the data files. This batch file contains pointers to the data files in the batch review itself. Upon loading a batch, the analyst need only select the method to use for the batch, and then individually select the desired data files to analyze in the batch. One can check the calibration accuracy, instrument performance and individual integrations before approving the results. Any chromatogram specific integration parameters which are modified can be stored with the data file for data traceability. This interactive environment provides complete access to all other data analysis capabilities, such as peak purity, library searching, etc., as well.

Batch review uses the same data analysis registers (ChromReg and ChromRes) as the standard data analysis and should therefore not be used in an online session that is currently performing analyses.

Batch Configuration

A batch is a user-selected series of data files that is processed using a user-defined method. All data files in the batch are processed using the same method. The processing steps carried out each time a new sample is loaded for review can be selected (integration, identification/quantitation, reporting).

All calibration runs in the batch are used to produce a single calibration table, using averaged response factors, which is then used for quantification.

Batch Table

Runs are displayed in a user-configurable batch table:

- the number and content of the table's columns can be specified;
- the runs can be sorted by
 - run index (the order in which the runs were acquired) independent of any other criteria,
 - sample type (control samples first, then calibration samples, then normal samples) then by run index within each sample type,
 - method (if more than one method was used to acquire the runs) then by run index within each method;
- samples, calibration samples and control samples can be displayed in the table or hidden.

Each selected run occupies a line in the batch table. You can disable the display of a run in the batch table by changing its sample type to Removed.

Compound Table

The compound results are displayed in a user-configurable compounds table, but contents of the compounds table depends on the type of samples in the batch table:

- the compound list contains all compounds found in the method that was loaded for batch review.
- if calibration samples only are displayed in the batch table (samples and

Batch Configuration

control samples are hidden), the compound table shows additional columns for calibration-related information (expected amount, relative error and absolute error).

- if control runs only are displayed in the batch table (samples and calibration samples are hidden), the compound table shows additional columns for any defined control limits.

For columns containing compound-specific information, you can include the name of the compound in the table title by adding %s to the column specification.

Batch Report

The batch report contains two tables that are generally analogous to the batch table and the compound table; these tables are also user-configurable.

For columns containing compound-specific information, you can include the name of the compound in the table title by adding %s to the column specification. Multi-line headers are allowed; you insert the character ‘|’ at the point where you want the line to break.

User Interface

Batch review provides a choice of two user interfaces:

- the standard interface includes a button bar, with buttons mirroring most of the Batch menu items, together with the batch table and compound table;
- a minimal interface provides a button bar similar to the standard interface, but replaces the batch table and compound table with a combo box that contains only the information specified for the batch table. The minimal interface button bar does not contain batch table-related or compound table-related buttons.

Review Functions

Data files can be displayed in one of two ways:

- manually, by selecting a run to display from the table,
- automatically, with a predefined interval between each data file. During automatic display, only those sample types displayed in the table are displayed; the runs are displayed in the order in which they appear in the table. The automatic review can be paused and later resumed, or stopped.

The standard functions provided by the ChemStation are available with batch review. This includes calibration, manual manipulation of chromatograms, for example by smoothing or manual integration. Any changes made to a data file can be marked and saved with the batch file. Chromatograms that have been reviewed are marked with an asterisk in the batch table. You can also discard changes made to either the current chromatogram only, or all changes to all chromatograms in the batch.

When a run is loaded, the selected processing options are performed; if the run has already been processed and the changes saved, the processed run is loaded. This is a faster process than loading the unprocessed run, because no processing needs to be done.

Batch Reporting

The user-configurable “Batch Table”, see page 220, can be printed directly on the printer, displayed on the screen or printed to a file with a user-specified prefix in one of the following formats:

- ASCII text file with the extension .TXT
- Data Interchange Format with the extension .DIF
- Comma-Separated Values format with the extension .CSV
- Microsoft Excel format with the extension .XLS.

The reporting options also give the possibility of sorting the samples (by Run Index, Sample Type or Method) independent of the sort method in the batch table. The sort priorities are as for the “Batch Table”, see page 220.

Batch History

Batch review maintains a log of all actions relating to the current batch. Any action that changes the batch (for example changing the displayed chromatogram, changing the sample type, loading and saving the batch) adds a line to the batch history with a date and time stamp, the current operator name and a description of the event.

You can also add your own comments to the batch history. Existing batch history entries cannot be edited, and the history list cannot be accessed except through the Batch History menu item.

Batch Review
Batch Reporting

**Using the ChemStation
Reports**

Using the ChemStation Reports

This chapter describes the following:

- what is a report?
- reporting results,
- quantitative results,
- report styles,
- report destination, and
- sequence summary 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.

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 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 the 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 Chapter 8 “Quantification”.

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.

Report Styles

The following report styles are available:

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

- **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).
- **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.

Report Styles

For uncalibrated methods, the report parameters include the peak number, retention/migration time, peak area, peak height, signal description, true half-height peak-width (see also “True Peak Width W_x [min]” on page 252), 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 peak width is calculated at the inflection points of the peak, it is not 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” on page 251 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 see “Performance Test Definitions” on page 251.

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 the 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” on page 84).

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, the 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 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.

Report Destination

The report can be sent to either:

- 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. If the data is saved to a file it is possible to reprocess the data with another program, for example, Microsoft Windows EXCEL.

Report File Formats

A report can be saved in four 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 an ASCII text file. |
| .WMF | 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. |

Report Destination

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.

Sequence Summary Reporting

Overview

The 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**

Sequence Summary Reporting

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 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” on page 239.

- **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:

Sequence Summary Reporting

retention/migration time,
area,
height,
amount,
peak width at half height, and
symmetry.

The statistic calculation does not distinguish between different calibration levels in a sequence that uses multilevel calibration methods. This means that the concentration-dependant 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 also define where the sequence summary report should be printed.

Selecting Report to file and entering a file name prints the report to the specified file. The default setting is that data are saved to the file GLPrpt.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.

Selecting Report to printer prints 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

Using the ChemStation Reports
Sequence Summary Reporting

these reports in the Sequence Output dialog or use the destination specified in the individual methods.

**Evaluating System
Suitability**

Evaluating System Suitability

Evaluating the performance of both the analytical instrument before it is used for sample analysis and the analytical method before it is used routinely is good analytical practice. It is also a good idea to check the performance of analysis systems before, and during, routine analysis. The ChemStation software provides the tools to do these three types of tests automatically. An instrument test can include the detector sensitivity, the precision of peak retention times and the precision of peak areas. A method test can include precision of retention times and amounts, the selectivity, and the robustness of the method to day-to-day variance in operation. A system test can include precision of amounts, resolution between two specific peaks and peak tailing.

Laboratories which have to comply with:

- Good Laboratory Practice regulations (GLP),
- Good Manufacturing Practice regulations (GMP) and Current Good Manufacturing Practice regulations (cGMP), and
- Good Automated Laboratory Practice (GALP)

are advised to perform these tests and to document the results thoroughly. Laboratories which are part of a quality control system, for example, to comply with ISO9000 certification, will have to demonstrate the proper performance of their instruments.

The ChemStation collates results from several runs and evaluates them statistically in the sequence summary report.

The tests are documented in a format which is generally accepted by regulatory authorities and independent auditors. Statistics include:

- peak retention time,
- peak area,
- amount,
- peak height,
- peak width at half height,
- peak symmetry,

- peak tailing,
- capacity factor (k'),
- plate numbers,
- resolution between peaks,
- selectivity relative to preceding peak,
- skew, and
- excess

The mean value, the standard deviation, the relative standard deviation and the confidence interval are calculated. You can set limits for either standard deviation, the relative standard deviation or the confidence interval for each of these parameters. Should the values exceed your limits, the report is flagged to draw your attention to them.

The quality of the analytical data can be supported by keeping records of the actual conditions at the time the measurements were made. The ChemStation's logbook records instrument conditions before and after a run. This information is stored with the data and reported with sample data. Instrument performance curves are recorded during the entire analysis as signals, and stored in the data file. If supported by the instrument these records, overlaid on the chromatogram/electropherogram, can be recalled on demand, for example, during an audit.

Baseline noise and drift can be measured automatically. A minimum detectable level can be calculated from peak height data for each calibrated compound in the method.

Finally, instrument configuration, instrument serial numbers, column identification, and your own comments can be included in each report printed.

Extended performance results are calculated only for compounds calibrated for in the method, ensuring characterization by retention times and compound names.

A typical system performance test report contains the following performance results:

- instrument details,
- column/capillary details,
- analytical method,
- sample information,
- acquisition information,
- signal description and baseline noise determination, and
- signal labeled with either retention/migration times, or compound names.

In addition, the following information is generated for each calibrated compound in the chromatogram/electropherogram:

- retention/migration time,
- k' ,
- symmetry,
- peak width,
- plate number,
- resolution,
- signal-to-noise ratio, and
- compound name.

Noise Determination

Noise can be determined from the data point values from a selected time range of a signal. Noise is treated in three different ways:

- 1 as six times the standard deviation (sd) of the linear regression of the drift,
- 2 as peak-to-peak (drift corrected), and
- 3 as determined by the ASTM method (ASTM E 685-93).

Figure 42

Noise as Six Times the Standard Deviation

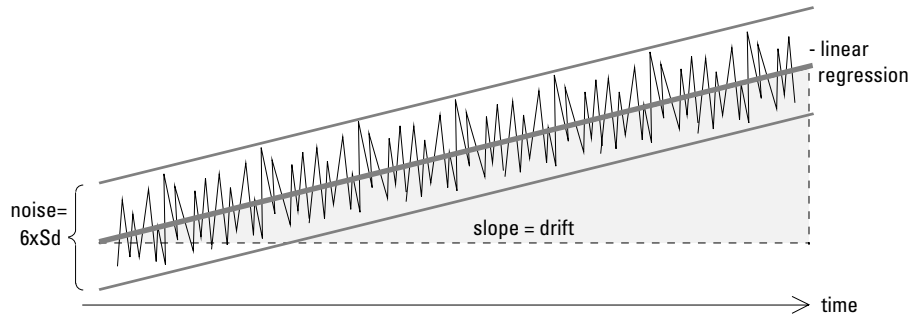
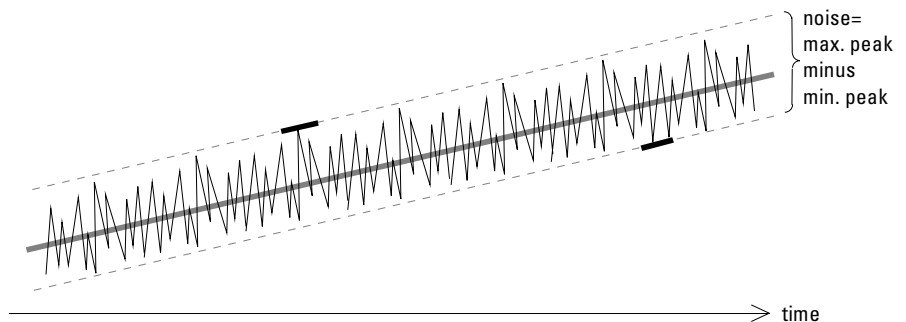


Figure 43

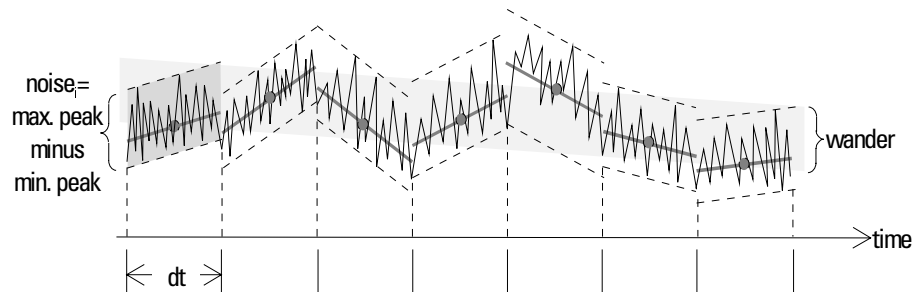
Noise as Maximum Peak to Minimum Peak (Distance)



Drift is given as the slope of the linear regression, see Figure 42, and wander is determined as the peak-to-peak noise of the mid-data values in the ASTM noise cycles, see Figure 44.

Figure 44

Noise Determined by the ASTM Method



ASTM noise determination (ASTM E 685-93) is based on the standard practice for testing variable-wavelength photometric detectors used in liquid chromatography, as defined by the American Society for Testing and Materials. Based on the size of the time range, three different types of noise can be distinguished. Noise determination is based on peak-to-peak measurement within defined time ranges.

- 1 Long-term noise—the maximum amplitude for all random variations of the detector signal of frequencies between 6 and 60 cycles per hour. Long-term noise is determined when the selected time range exceeds one hour. The time range for each cycle (dt) is set to 10 minutes which will give at least six cycles within the selected time range.
- 2 Short-term noise—the maximum amplitude for all random variations of the detector signal of a frequency greater than one cycle per minute. Short-term noise is determined for a selected time range between 10 and 60 minutes. The time range for each cycle (dt) is set to one minute which will give at least 10 cycles within the selected time range.
- 3 Very-short-term noise (not part of ASTM E 685-93)—this term is introduced to describe the maximum amplitude for all random variations of the detector signal of a frequency greater than one cycle per 0.1 minute. Very-short-term noise is determined for a selected time range between 1 and 10 minutes. The time range for each cycle (dt) is set to 0.1 minute which will give at least 10 cycles within the selected time range.

An ASTM noise determination is not done if the selected time range is below one minute. Depending on the range, if the selected time range is greater than, or equal to one minute, noise is determined using one of the ASTM methods previously described. At least seven data points per cycle are used in the calculation. The cycles in the automated noise determination are overlapped by 10 %.

Calculation of Peak Symmetry

The ChemStation does not determine the asymmetry ratio of a peak, usually done by comparing the peak half-widths at 10% of the peak height, or 5% as recommended by the FDA.

Peak symmetry is calculated as a pseudomoment by the integrator using the following moment equations:

$$m_1 = a_1 \left(t_2 + \frac{a_1}{1.5H_f} \right)$$

$$m_2 = \frac{a_2^2}{0.5H_f + 1.5H}$$

$$m_3 = \frac{a_3^2}{0.5H_f + 1.5H}$$

$$m_4 = a_4 \left(t_3 + \frac{a_4}{1.5H_f} \right)$$

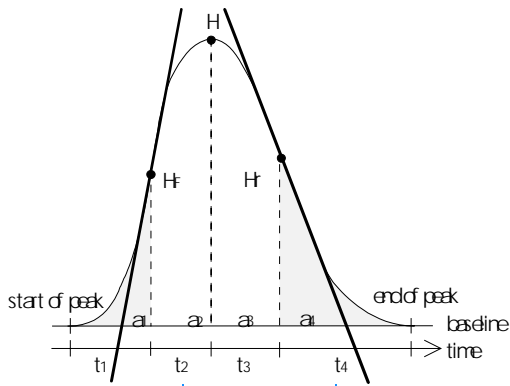
$$\text{Peak symmetry} = \sqrt{\frac{m_1 + m_2}{m_3 + m_4}}$$

If no inflection points are found, or only one inflection point is reported, then the peak symmetry is calculated as follows:

$$\text{Peak symmetry} = \frac{a_1 + a_2}{a_3 + a_4}$$

Figure 45

Calculation of the Peak Symmetry Factor



where:

a_i = area of slice

t_i = time of slice

H_f = height of front inflection point

H_r = height of rear inflection point

H = height at apex

System Suitability Formulae and Calculations

The ChemStation uses the following formulae to obtain the results for the various System Suitability tests. The results are reported using the Performance, Performance + Noise and Performance + Extended report styles.

When ASTM or USP is specified for a given definition, then the definition conforms to those given in the corresponding reference. However, the symbols used here may not be the same as those used in the reference.

The two references used in this context are:

- 1** *ASTM: Section E 682 – 93, Annual Book of ASTM Standards, Vol.14.01*
- 2** *USP: The United States Pharmacopeia, XX. Revision, pp. 943 - 946*

General Definitions

Void Volume

$$V = d^2\pi l(f/4)$$

where:

d = diameter of column [cm]

π = constant, ratio of circumference to diameter of a circle

l = length of column [cm]

f = fraction of column volume that is not taken up by stationary phase but available for mobile phase; default value for f = 0.68 (for Hypersil)

Retention Time of Unretained Compound t_m [min]

(Also referred to as dead time or void time)

$$T_m = V/F$$

where:

F = flow rate of LC [ml/min]

Performance Test Definitions

Statistical Moments

$$M0 = d_t \cdot X$$

$$M1 = t_0 + d_t \cdot \frac{X}{Y}$$

$$M2 = \frac{d_t^2}{X} \cdot \sum_{i=1}^N \left(\left(i - 1 - \frac{Y}{X} \right)^2 \cdot A_i \right)$$

$$M3 = \frac{d_t^3}{X} \cdot \sum_{i=1}^N \left(\left(i - 1 - \frac{Y}{X} \right)^3 \cdot A_i \right)$$

$$M4 = \frac{d_t^4}{X} \cdot \sum_{i=1}^N \left(\left(i - 1 - \frac{Y}{X} \right)^4 \cdot A_i \right)$$

where:

N = number of area slices

A_i = value of area slice indexed by i

$\sum_{i=1}^N$ = sum of starting index 1 to final index N for discrete observations

d_t = time interval between adjacent area slices

t₀ = time of first area slice

$X = \sum_{i=1}^N (A_i)$

$Y = \sum_{i=1}^N ((i-1)A_i)$

Statistical Moments, Skew and Excess

Statistical moments are calculated as an alternative to describe asymmetric peak shapes. There is an infinite number of peak moments, but only the first five are used in connection with chromatographic peaks.

These are called **0th Moment**, **1st Moment**, ... **4th Moment**.

The **0th Moment** represents the peak area.

The **1st Moment** is the mean retention time, or retention time measured at the center of gravity of the peak. It is different from the chromatographic retention time measured at peak maximum unless the peak is symmetrical.

The **2nd Moment** is the peak variance which is a measure of lateral spreading. It is the sum of the variance contributed by different parts of the instrument system.

The **3rd Moment** describes the vertical symmetry or skew. It is a measure of the departure of the peak shape from the Gaussian standard. The skew given additionally in the Performance & Extended report is its dimensionless form. A symmetrically peak has a skew of zero. Tailing peaks have positive skew and their 1. Moment is greater than the retention time. Fronting peaks have negative skew and their 1. Moment is less than the retention time.

The **4th Moment** or excess is a measure of the compression or stretching of the peak along a vertical axis, and how this compares to a Gaussian standard for which the 4. Moment is zero. It can be visualized by moving in or pulling apart the sides of a Gaussian peak while maintaining constant area. If the peak is compressed or squashed down in comparison, its excess is negative. If it is taller, its excess is positive. Also the excess is given in the Performance & Extended report in its dimensionless form.

True Peak Width W_x [min]

W_x = width of peak at height x % of total height

Special cases:

W_B	base width, 4 sigma, obtained by intersecting tangents through the inflection points with the baseline (<i>tangent peak width</i>) see Figure 46 on page 253)
$W_{4.4}$	width at 4.4% of height (<i>5 sigma width</i>)
$W_{5.0}$	width at 5% of height (<i>tailing peak width</i>), used for USP tailing factor

$W_{50.0}$ width at 50% of height (*true half-height peak width* or *2.35 sigma*). See Figure 46 for details.

Capacity Factor (USP), Capacity Ratio (ASTM) k'

$$k' = \frac{T_R - T_0}{T_0}$$

where:

T_R = retention time of peak [min]

T_0 = void time [min]

USP Tailing Factor (USP) t

$$t = \frac{W_{5.0}}{t_w \cdot 2}$$

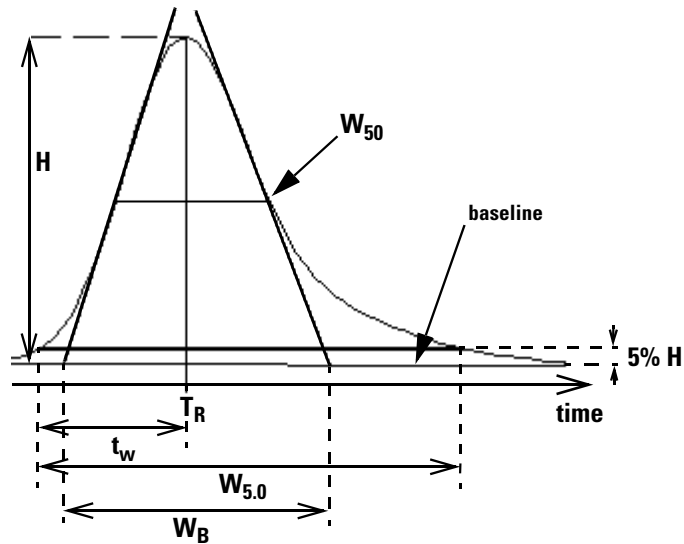
where:

t_w = distance in min between peak front and T_R , measured at 5% of the peak height (see Figure 46)

$W_{5.0}$ = peak width at 5% of peak height [min]

Figure 46

Performance Parameters



Number of Theoretical Plates per Column (USP, ASTM) n

Tangent method (USP, ASTM):

$$n = 16 \left(\frac{T_R}{W_B} \right)^2$$

where:

W_B = base width [min] (see Figure 46 on page 253)

Half-width method (ASTM):

$$n = 5.54 \left(\frac{T_R}{W_{50}} \right)^2$$

where:

W_{50} = peak width at half-height [min] (see Figure 46 on page 253)

5 Sigma method:

$$n = 25 \left(\frac{T_R}{W_{4.4}} \right)^2$$

where:

$W_{4.4}$ = peak width at 4.4% of peak height [min]

Variance method:

$$n = \frac{M_1^2}{M_2}$$

where:

M_x = x th statistical moment (see also “Statistical Moments” on page 251)

Number of Theoretical Plates per Meter N [1/m]

$$N = 100 \frac{n}{l}$$

where:

n = number of theoretical plates

l = length of column [cm]

Relative Retention (USP, ASTM), Selectivity Alpha

(Pertaining to peaks a and b, T_R of peak a < T_R of peak b)

$$\alpha = \frac{k'_{(b)}}{k'_{(a)}}, \alpha \geq 1$$

where:

$$k'_{(x)} = \text{capacity factor for peak } x$$

Resolution (USP, ASTM) R

(Pertaining to peaks a and b, T_R of peak a < T_R of peak b; T_R in min)

Tangent method (USP, ASTM):

$$R = \frac{2(T_{R(b)} - T_{R(a)})}{W_{B(b)} + W_{B(a)}}$$

5 Sigma method:

$$R = \frac{2.5(T_{R(b)} - T_{R(a)})}{W_{4.4(b)} + W_{4.4(a)}}$$

Half-width method:

$$R = \frac{(2.35/2)(T_{R(b)} - T_{R(a)})}{W_{50(b)} + W_{50(a)}}$$

Statistical method:

$$R = \frac{M1_{(b)} - M1_{(a)}}{W_{S(b)} + W_{S(a)}}$$

where:

$M1_{(x)}$ = mean retention time for peak x (1st Statistical Moment) [min]

$W_{B(x)}$ = base width for peak x [min]

$W_{4.4(x)}$ = width at 4.4% height for peak x [min]

$W_{50(x)}$ = width at 50% height for peak x [min]

$W_{S(x)}$ = width derived from statistical moments = $\sqrt{(M2)}$ for peak x
(see also "Statistical Moments" on page 251) [min]

Definitions for Reproducibility

For the statistical review of analytical data in terms of reproducibility the sequence is considered as a small random sample taken out of an infinite number of possible experimental results. To accomplish a complete set of results, an unlimited amount of sample material as well as time would be required. Strictly statistical data does only apply to a complete self-contained set or population of data. Therefore a prerequisite for such a treatment is that the selected sample can be assumed as representative for all data.

Sample Mean M

The mean value M of a random sample consisting of N measurements is calculated from this limited set of N single observed values X_i indexed with a consecutive counter i according to the formula:

$$M = \frac{\sum_{i=1}^N X_i}{N}$$

where:

N = number of discrete observations

X_i = value of discrete observations indexed by i

Sample Standard Deviation S

Consider a random sample of size N. The sample standard deviation S for the selected finite sample taken out of the large population of data is determined by

$$S = \sqrt{\frac{\sum_{i=1}^N (X_i - M)^2}{N - 1}}$$

The sample standard deviation S differs in two points from the standard deviation s for the whole population:

- instead of the real mean value only the sample mean value M is used and
- division by $N-1$ instead of N .

Relative Standard Deviation RSD[%] (USP)

The relative standard deviation is defined as

$$\text{RSD} = 100 \frac{S}{M}$$

Standard Deviation of the Mean S_M

Let M be the sample mean and S the sample [or $(N-1)$] standard deviation. The standard deviation S_M of the sample mean M is determined by

$$S_M = \frac{S}{\sqrt{N}}$$

Example

This can be further illustrated by an example:

While the retention time of a certain compound may deviate slightly from the calculated mean value during one sequence, the data from another sequence may differ much more due to e.g. ambient temperature changes, degradation of the column material over time etc. To determine this deviation the standard deviation of the sample mean S_M can be calculated according to the above formula.

Confidence Interval CI

The confidence interval is calculated to give information on how good the estimation of a mean value is, when applying it to the whole population and not only to a sample.

The $100 \cdot (1 - \alpha) \%$ confidence interval for the overall mean is given by

$$\text{CI} = t_{(\alpha/2); N-1} \cdot S_M$$

where:

$t_{(\alpha/2); N-1}$ percentage point of the t distribution table at a risk probability of α

Definitions for Reproducibility

For the extended statistics in the sequence summary report the 95% confidence interval may be used ($\alpha = 0.05$).

The t distribution (or ‘student distribution’) must be used for small sample volumes. In case of large sample volumes the results for the t distribution and the normal (gaussian) distribution do not differ any more. Therefore in case of 30 or more samples the normal distribution can be used instead (it would be very difficult to calculate the t-distribution for large numbers, the normal distribution is the best approximation of it).

Example

95% Confidence Interval for 6 samples:)

$$1 - \alpha = 0.95$$

$$N = 6$$

The correct value for t has to be taken from the t distribution table for 5 (N-1) degrees of freedom and for the value $\alpha/2$, being 0.025. This would give the following calculation formula for CI:

$$CI = 2.571 \cdot \frac{1}{\sqrt{6}} \cdot S_M$$

Regression Analysis

Let

N = number of discrete observations

X_i = independent variable, i^{th} observation

Y_i = dependent variable, i^{th} observation

Linear function: $y_{(X)} = a + bX$

Coefficients:

$$a = \frac{1}{\Delta_x} \left(\sum_{i=1}^N X_i^2 \cdot \sum_{i=1}^N Y_i - \left(\sum_{i=1}^N X_i \cdot \sum_{i=1}^N X_i Y_i \right) \right)$$

$$b = \frac{1}{\Delta_x} \left(N \cdot \sum_{i=1}^N X_i Y_i - \left(\sum_{i=1}^N X_i \cdot \sum_{i=1}^N Y_i \right) \right)$$

where:

$$\Delta_X = N \cdot \sum_{i=1}^N X_i^2 - \left(\sum_{i=1}^N X_i \right)^2$$

Regression Coefficient

$$r = \frac{\left(N \cdot \sum_{i=1}^N X_i Y_i - \sum_{i=1}^N X_i \cdot \sum_{i=1}^N Y_i \right)}{\sqrt{\Delta_X \cdot \Delta_Y}}$$

where:

$$\Delta_Y = N \cdot \sum_{i=1}^N Y_i^2 - \left(\sum_{i=1}^N Y_i \right)^2$$

Standard Deviation (S)

$$S = \sqrt{\frac{\sum_{i=1}^N (Y_i - a - bX_i)^2}{N - 2}}$$

Internally Stored Double Precision Number Access

For validation purposes, it might become necessary to manually recalculate the ChemStation results such as calibration curves, correlation coefficients, theoretical plates, etc. When doing so the number format used in the ChemStation has to be taken into account.

For all numbers stored internally within the ChemStation, the “C” data type DOUBLE is used. This means that 14 significant digits are stored for each number. The implementation of this data type adheres to the Microsoft implementation of the IEEE standard for “C” data type and the associated rounding rules (see Microsoft documents Q42980, Q145889 and Q125056).

Due to the non-limited number of parameters that might be used for the calculation of the calibration table, it is not possible to calculate the exact error possibly introduced by the propagation and accumulation of rounding errors. Thorough testing with different calibration curve constructions however has shown that the accuracy of up to 10 digits can be guaranteed. Whereas the area, height and retention time repeatability of a chromatographic analysis usually has 3 significant digits, 10 significant digits within the calculations is sufficient. For this reason, the calibration, and other tables, display a maximum of 10 significant digits.

If an external (manual) calculation for validation is required, it is recommended that all digits used for the internal calculations are utilized. Using the displayed and/or rounded data for the external calculations might give results differing from the ChemStation due to rounding errors.

The following paragraph describes how to access all internally stored digits for numbers typically required for manual calculations. In all cases, a data file must be loaded and reported with the appropriate report style prior to execution of the listed command. All commands are entered on the ChemStation command line which may be enabled from the view menu. The information in file “C:\HPCHEM\TEMP.TXT” may be viewed using NOTEPAD or a suitable TEXT editor.

Raw Peak Information:

- Retention Time
- Area

- Height
- Width (integrator)
- Symmetry
- Peak Start Time
- Peak End Time

Use Command Line Entry:

```
DUMPTABLE CHROMREG, INTRESULTS, "C:\HPCHEM\1\TEMP\INTRES.TXT"
```

Processed Peak Information:

- Measured Retention Time
- Expected Retention Time
- Area
- Height
- Width (integrator)
- Symmetry
- Half Width - Half Peak Height (Performance & Extended Performance)
- Tailing Factor (Performance & Extended Performance)
- Selectivity (Performance & Extended Performance)
- K' (Extended Performance)
- Tangent Peak Width (Extended Performance)
- Skew (Extended Performance)
- Theoretical Plates - Half Width (Performance & Extended Performance)
- Theoretical Plates - Tangent (Extended Performance)
- Theoretical Plates – 5-Sigma (Extended Performance)
- Theoretical Plates - Statistical (Extended Performance)
- Resolution - Half Width (Performance & Extended Performance)
- Resolution - Tangent (Extended Performance)
- Resolution – 5-Sigma (Extended Performance)
- Resolution - Statistical (Extended Performance)

Use Command Line Entry:

```
DUMPTABLE CHROMRES, PEAK, "C:\HPCHEM\1\TEMP\PEAK.TXT"
```

Processed Compound Information:

- Calculated Amount

Use Command Line Entry:

```
DUMPTABLE CHROMRES, COMPOUND, "C:\HPCHEM\1\TEMP\COMPOUND.TXT"
```

Calibration Table Information:

- Level Number
- Amount
- Area
- Height

Use Command Line Entry:

```
DUMPTABLE _DAMETHOD, CALPOINT, "C:\HPCHEM\1\TEMP\CALIB.TXT"
```

Linear Regression Information:

- Y-Intercept (CurveParm1)
- Slope (CurveParm2)
- Correlation Coefficient

Use Command Line Entry:

```
DUMPTABLE _DAMETHOD, PEAK, "C:\HPCHEM\1\TEMP\REGRESS.TXT"
```

System Verification

System Verification

System Verification

System verification is a key component in the routine use of an analytical instrument in a regulated laboratory. The GLP verification features of the ChemStation are designed to help you to prove that the software, or a relevant component of the software, are performing correctly, or were performing correctly at the time of a particular analysis.

The ChemStation verification function enables you to verify the correct operation of your ChemStation software. You can do this by reprocessing data files according to specific methods, and comparing the results with a pre-defined standard. The verification function is particularly important to prove the integrity of the integration and quantification results.

You can use the standard verification test, or define your own tests using your own method and data files to check the algorithmic software combinations used by your analysis methods. The verification test is a protected file and cannot be changed or deleted.

The Verification item in the Data Analysis view allows you to choose any of the following options:

- run a verification test in the database,
- define a new verification test and add it to the database, and
- delete a verification test from the database.

The How To section of the online help system describes how to perform these tasks. When running a ChemStation verification test, you can choose whether to run the entire test, or select a combination of parts.

Verification test results are saved in binary format to the default subdirectory: c:\hpchem\1\Verify, together with the method and data files. The Verify subdirectory is at the same level as the sequence, methods and data subdirectories. You can send the results to a printer or to a file. The test results, including a combined verification test result, are rated as either pass or fail.

The following verification test components are available:

Digital Electronics (HP 1050 DAD and Agilent 1100 Series DAD only)

A test chromatogram is stored in the diode-array detector. This chromatogram is sent to the ChemStation after it has gone through the same preprocessing steps as normal raw data from the photodiodes. The resulting data are compared to original result data stored in the ChemStation for this test chromatogram. If there is a mismatch the test fails. This test ensures that the DAD electronics which do the data preprocessing are still functioning correctly. As a stored test chromatogram is used, the lamp or the diode array are not part of this test. They can be checked with the “DAD Test Function” on page 269.

Peak Integration

The data file is integrated again using the original method. The results are compared to the original integration results stored in verification register. If they do not match, the test fails.

Compound Quantification

The compounds in the data file are quantified again. The results are compared to the original quantification results stored in the verification register. If they do not match, the test fails.

Report Printing

The original report is printed again.

The following page shows an example of a successfully completed verification test.

System Verification

System Verification

```
=====
                        ChemStation Verification Test Report
=====
Tested Configuration:
  Component                Revision                Serial Number
-----
  HPLC 2D ChemStation      A.05.01                N/A
  Microsoft Windows        3.95 (enhanced mode)   N/A
  MS-DOS                    7.0                    N/A
  Processor                 Pentium                 N/A
  CoProcessor               yes                     N/A
ChemStation Verification Test Details:
  Test Name                 : C:\HPCHEM\1\VERIFY\DEFAULT.M
  Original Data File        : C:\HPCHEM\1\VERIFY\DATA\NV-0019.D
  Original Acquisition Method : TC1ISO2.M
  Original Data Analysis Method : TC1ISO2.M
  Original Operator         : Hewlett-Packard
  Original Injection Date   : WED Feb 17 08:59:51 1997
  Original Sample Name     :
Signals Tested:
  Signal 1: DAD1 A, Sig=254,4 Ref=450,80 of NV-0019.D
  Signal 2: VWD1 A, Wavelength=254 nm of NV-0019.D
ChemStation Verification Test Results:
  Test Module              Selected For Test      Test Result
-----
  Digital electronics test No                       N/A
  Integration test         yes                     Pass
  Quantification test      yes                     Pass
  Print Analytical Report  No                       N/A

ChemStation Verification Test Overall Results:      Pass
```

Verification and Diagnosis Views

If supported by the configured instrument, for example, the Agilent 1100 Series modules for LC, the ChemStation comprises two additional views to perform instrument verification and diagnosis tasks. For more information, see the online help system and the *Performance Verification* manual.

The GLPsave Register

The GLPsave register is saved at the end of each analysis when selected in the run time checklist. It contains the following information:

- signals,
- logbook,
- integration results table,
- quantification results table,
- instrument performance data, and
- data analysis method.

This register is a complete protected record, generated at the time of analysis. You can recall it at any time in the future as proof of your analytical methods.

The GLPsave Register option in the Data Analysis view enables you to review the GLPsave register file at any time. The file is protected by a checksum and is encoded into binary to ensure it is not changed.

In the dialog box used to select the GLPsave register for review, you can choose your review options from the following:

- load original method,
- load original signals,
- load instrument performance data,
- print original method,
- print original integration results,
- print original quantification results, and
- generate original report from the original method and signals.

You can use the GLP review function to show that chromatographic data are original, prove the quality of the analysis from the instrument performance data, and demonstrate the authenticity of the data interpretation.

For example, you can:

- reload and reprint the data analysis part of the method used at the time of

the sample analysis to prove that the data evaluation, presented as the results of the analysis has not been modified in any way, and

- review without recalculating, the integration and quantification results to prove the authenticity of the report.

DAD Test Function

Detector tests can be used as a step in the routine system validation of an analytical instrument in a regulated laboratory.

The DAD test assesses the performance of your diode array detector. When you select the DAD test from the Instrument menu (for LC3D on 3DCE-only) it checks the instrument for intensity and wavelength calibration. When you press Save the test results are automatically saved to the DADTest database, a register file called DADTest.Reg located in the default instrument directory.

Review DAD Test Function

The Review DAD Test function in the data analysis View menu enables you to review the DADTest.Reg file at any time. The file is protected by a checksum, and is encoded into binary to ensure that it is not changed.

You can select any of the following parts of the DAD test for review:

Show Holmium Spectra

Plots all Holmium spectra listed in the DAD Test review table. The active spectrum is tagged.

Show Intensity Spectra

Plots all intensity spectra listed in the DAD Test review table. The active spectrum is tagged.

Save as New Database

If you change the lamp in your DAD you can reset the DADTest by deleting any unwanted test results from the table and then using the Save As New Database function.

Show Selected Spectra

Displays only spectra you selected in the table.

Show Intensity Graph

You can plot an intensity graph to give an indication of the life of the lamp in your diode array detector. The graph provides a function of maximum lamp intensity against time.

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Agilent Technologies

In This Book

This handbook describes various concepts of the Agilent ChemStation. It is intended to increase your understanding of how the ChemStation works.

For information on using the ChemStation please refer to the help system, especially the How To section, and the integrated tutorial.



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