Agilent ChemStation for GC, LC, LC/MSD, CE, and A/D Systems– Rev. 09.0x

Specifications

What's new in A.09.01

Revision A.09.01 replaces revision A.08.0x. Simliar to past revisions, the customers currently operating an Agilent ChemStation with a GC, LC, LC/MSD, CE or A/D instrument can take the advantage of the new features by upgrading their software. The following new features are part of revision A.09.01:

General Description

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

- Agilent 5890 Series II, Agilent 6890 and 6850 Series gas chromatographs,
- Agilent 1100 Series modules and systems for HPLC, including the Agilent 1100 Series LC/MSD,
- HP 1090 Series liquid chromatographs,
- HP 1050 Series liquid chromatography modules,
- Agilent Capillary Electrophoresis systems, and
- Agilent 35900E dual channel analog-to-digital interfaces.

The software is designed to run on IBM compatible personal computers with an ISA or PCI interface bus under Microsoft® Windows

- Support for LC & LC/MS Purification system
- Support for APPI source in LC/MS
- Support for new 1100 modules:
  - Fraction collector (G1364A)
  - Preparative autosampler (G2260A/G2261A)
  - Multiple purpose switching valve (G1160A)
  - Universal interface box (G1390A)
- New IP-Address management

operating environments. The software is sold as single instrument basic ChemStations in six 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 six versions are
- a single instrument Agilent ChemStation for gas chromatography (GC) systems, product number G2070AA,
- a single instrument Agilent ChemStation for liquid chromatography (LC) systems, product number G2170AA,

for 1100 systems
- Support for Windows 2000 Service Pack 2 and Windows NT 4.0 Service Pack 6a
- Defect fixes

For an overview of the defects that have been fixed with revision A.09.01, please review the ChemStation Revision History (directory A_09_01 in support/history on the ChemStation CD-ROM).

- a single instrument Agilent Chem-Station for liquid chromatography/mass selective detector (LC/MSD) systems, product number G2710AA,
- a single instrument Agilent Chem-Station 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.
- CE/MS Add-on software, product number G1946X.

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

∴ **Agilent Technologies**

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,
- additional analog data acquisition module, product number G2073AA
- additional CE/MSD add-on software, product number G2201AA for G1946X

Additional Data Evaluation Modules

The data evaluation capability of the ChemStations may also be expanded through the purchase of additional data evaluation modules for specialist applications:
- additional diode-array detector (DAD) spectral evaluation module, product number G2180AA,
- additional Agilent ChemStore sample organization and resultsdatabase module, product number G2181BA,
- additional Agilent ChemStation Plus Security Pack, product number G2183AA,
- additional Agilent ChemStation Plus Method Validation Pack, product number G2184AA,
- 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 Agilent ChemStation, however the total number of modules is limited for multiple instrument configurations. An example of an allowed instrument configuration for four instruments on one Agilent ChemStation is:
- 1 variable wavelength detector
- 1 autosampler
- 1 pump.

Four such instruments are supported on one Agilent ChemStation. An example of a configuration for a spectral detector system would be:
- 1 diode array detector
- 1 autosampler
- 1 pump
- 1 thermostatted column compartment.

Two such instruments are supported on one Agilent ChemStation. For specific configurations of multiple instruments on Agilent ChemStations, please contact your Agilent Technologies service or sales representative.

Data Evaluation-only Products

There are also three data evaluation-only products that may not have instruments configured. They are designed to be used for data evaluation in an office environment:
- The Agilent ChemStation for data evaluation, product number G2090AA, has the same data evaluation capabilities as the basic Agilent ChemStations.
- The Agilent ChemStation for LC 3D data evaluation, product number G2190AA, includes diode-array spectral data evaluation and the capabilities of the Agilent ChemStation for data evaluation.
- The Agilent 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 Agilent ChemStation for data evaluation.

Computer Hardware

The ChemStation consists of Hewlett-Packard personal computer hardware and ChemStation software. The hardware is an IBM compatible personal computer with an ISA or PCI interface bus.

The personal computer is interfaced to the analytical instruments through a LAN card, HP 82341C/D, HP 82350 GP-IB internal card, or a combination of one GP-IB card and a LAN card. All cards plug directly into the computer's PCI or ISA interface bus. Third-Party instruments can be connected via the Agilent 35900E A/D-Converter interface. The separate hardware components that comprise a particular instrument configuration, including third party instrumentation, may need to be coordinated through a remote cabling system for time critical events such as injection.

The ChemStation software has been tested on Agilent Technologies ChemStation (PC) hardware that conforms to the above specifications. Although the software is also designed to be run on other IBM-Pentium PCs compatible hardware, Agilent Technologies will not necessarily accept responsibility for defects reported on third party hardware.

Minimum PC Configuration

Windows NT 4.0 based systems:
- HP Vectra Pentium 266 MHz
- Super VGA display (800 x 600 resolution)
- 4 GB hard disk
- CD-ROM drive

Minimum memory specifications:
- 64 MB RAM for a 2D single instrument configuration,
- plus 8 MB RAM for every additional instrument configured,
- 96 MB RAM for a 3D single instrument configuration (LC, LC/MSD or CE),
- plus 8 MB RAM for every additional application such as the Agilent ChemStore database application.

Windows 2000 based systems:
- HP Vectra Pentium 266 MHz
- Super VGA display (800 x 600 resolution)
- 4 GB hard disk
- CD-ROM drive

Minimum memory specifications:
- 96 MB RAM for a 2D single instrument configuration,
- plus 8 MB RAM for every additional instrument configured,
- 128 MB RAM for a 3D single instrument configuration (LC, LC/MSD or CE),
- plus 8 MB RAM for every additional applications such as the Agilent ChemStore database.

Maximum Instrument Configurations

A maximum of four instruments may be interfaced to each Agilent ChemStation. However, the total number of modules allowed in a four intrument cluster is limited: *one VWD, one pump and one autosampler only.*
For proper configuration of specific instruments, please contact your Agilent Technolgies representative. Specific limitations apply:

- Only two diode array detector instruments (LC or CE) may be interfaced per PC system.
- When using Agilent ChemStore database software, a maximum of three instruments are supported on the PC system
- When an Agilent ChemStation is used to control the Agilent 1100 Series LC/MSD module (optionally with one Agilent 1100 Series or HP 1090 Series II HPLC), no additional instruments are supported on the PC system.

- When an Agilent ChemStation is used to control the Agilent 1100 Series LC/MSD module, in conjuction with the Agilent Capillary Electrophoresis system, no additional instruments are supported on the PC system.

Recommended PC Configuration

Windows NT or Windows 2000 based systems
- HP Vectra VL 800 Pentium IV 1,5 GHz (single instrument or multiple instrument configurations)
- Super VGA display (at 1024 x 768 resolution)
- 20 GB hard disk
- CD-ROM drive

Minimum memory specifications:
- Single instrument configuration: 128 MB RAM
- Multiple instrument, Agilent ChemStore or 3D configuration: 128 MB RAM
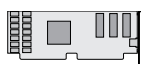
Note:

If you plan to install Windows NT 4.0 or Windows 2000, all PC hardware and peripherals must be listed in the Microsoft Windows NT Hard-ware Compatibility List (HCL), available from Microsoft under http://www.microsoft.com/hcl/

IEEE-488 GP-IB Support Matrix
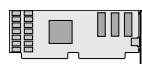
**82341C or D
IEEE-488 Card**

82350 GP-IB Card

- **Windows NT 4.0/Windows 2000**
  Supports all instruments except
  Agilent 1100 Series LC/MSD
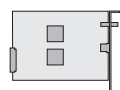 (G1946B/C/D models).

- **Windows NT 4.0/Windows 2000**
  Supports all instruments except
  Agilent 1100 Series LC/MSD
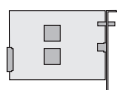 (G1946B/C/D models).

Note:
- *GP-IB communication
  requires to install the SICL
  I/O library version J.02.00.01
  (Please ask your local Agilent
  Technologies representative
  for details).*

LAN-MIO Support Matrix

**J2552B JetDirect
Internal Printer
Servers (MIO)**

**J4100 JetDirect N
Internal Printer
Servers (MIO)**

- Control instrument over LAN.
- Supports Agilent 6890 GC,
  Agilent 1100 Series LC, Agilent
  1100 Series LC/MSD, and
  35900E A/D converters.
- Control instrument over LAN
  using industry standard TCP/IP
  (Transmission control
  protocol/internet protocol).
- The firmware of the JetDirect
  card has to be revision A.05.05
  or higher.

- Control instrument over LAN.
- Supports Agilent 6890 GC,
  Agilent 1100 Series LC, Agilent
  1100 Series LC/MSD, and
  35900E A/D converters.
- Control instrument over LAN
  using industry standard TCP/IP
  (Transmission control proto-
  col/internet protocol).

Note:
- *LAN communication with the
  Agilent 6890 requires
  firmware revision 3.05 or
  higher. This is available in
  an electronic chip format
  from Agilent. Technologies
  (Please ask your local Agilent
  Technologies representative for
  details.)*
- *LAN communication with the
  Agilent 1100 Series requires
  firmware revision A.05.01 or
  greater. This is available on
  the Agilent Chem-Station CD-
  ROM and can be updated
  quickly and easily. (Please ask
  your local Agilent Technologies
  representative for details.)*

LAN/RS 232 Support

**G2403A
LAN/RS232 converter box**

- Control serial instruments over
  the LAN
- Supports Agilent 5890 Series II
  GC and the G1512A ALS con-

LAN Configuration Requirements

Instruments Supported on LAN
- Agilent 6890 gas chromatograph
- Agilent 6850 gas chromatograph
- Agilent 1100 Series LC systems
- Agilent 1100 Series LC/MSD
- Agilent 35900E A/D converter

LAN communication can be used with the Agilent 6890 and both Agilent 7683 and Agilent 7673. The Agilent 7673 communicates with the Agilent 6890 via RS232 cables from the GC instrument.

Instrument Firmware Requirements
- For the Agilent 6890, firmware revision 3.05 or greater is required in chip format.
- For the Agilent 1100 Series, firmware revision A.05.01 is recommended and is available on the ChemStation software CD-ROM.

Protocol Supported
- The protocol used for instrumentcontrol is the industry standard TCP/IP (transmission control protocol/internet protocol). This protocol needs to be loaded and configured in Windows NT 4.0 or Windows 2000 to support instrument control using LAN cables.

- For Agilent 1100 Series LC/MSD (G1946A systems) Revision A.3.01.69 is required for smart card 2.

Additional Hardware Required for LAN Instrument Control
- Industry standard LAN card for PC:
  Agilent instrument bundles ship with this standard.

- Instrument LAN card:
  This card can be ordered from Agilent Technoligies as an option to the instrument or standalone. Please see your Agilent Technologies representative for details.

- Industry standard LAN cabling using twisted pair or coaxial cabling:
  Twisted pair
  - 10/100 baseT twisted pair with RJ45 connectors can be used with a Agilent G2402A analytical hub for the ability to connect one, or more than one, instrument to the PC.
  - A twisted pair *'crossover wire'* can be used for making a single connection from one PC to one instrument. This configuration will only work in single instrument situa tions *(not supported on the Agilent 1100 Series LC/MSD systems)*.

- 10base2 coax cabling with the appropriate tee connectors and terminators can be used as well, when configuring single and multi-instrument systems.

- LAN transmission rates
  Traffic on the LAN from each instrument is ~100Kbps for a 2D instrument at maximum data rate.

LC/MSD ChemStation Computer Hardware

The LC/MSD ChemStation has a distinct set of hardware specifications and requirements. The software requires data system components with the following minimal specifications:

- An IBM compatible personal computer equipped with an Intel Pentium processor operating at 350 MHz or greater.

- At least 128 MB RAM.
- A hard disc drive with at least 4 GB capacity.
- A Microsoft Windows compatible pointing device.
- An ISA or PCI interface bus for the data acquisition interface.
- A super VGA or ultra VGA monitor and interface. The screen resolution must be at least 800 x 600. To take full advantage of the ChemStation's graphical user interface for LC/MS data analysis 1024 x 768 is recommended.
- A 10/100 baseT LAN interface card.
- A CD-ROM drive (4x speed or better).
- Audio card or integrated audio capability.
- HP LaserJet 5 with 4 MB RAM.

Printers

The software operates with any Microsoft Windows compatible printer directly connected to the computer through a parallel or serial interface or a Microsoft Windows compatible printer connected through a Local Area Network. The recommended black and white printers are the HP LaserJet family or, for lower performance applications, the HP DeskJet family. For LC applications, the recommended color printer is the HP DeskJet family which is capable of interpreting an escape code language (e.g. PCL) or page description language (e.g. Postscript). Host-based printers (e.g. GDI printers) impose more processing tasks on the CPU and are not recommended for use with the Agilent ChemStation. Also note that the DeskJet printer family is not recommended for high throughput applications.

Operating System

The Agilent ChemStation requires Microsoft Windows NT 4.0 operating system with Service Pack 6a or Windows 2000 with Service Pack 2.

Methods and Sequences

The Agilent ChemStation analytical method fully describes how a particular separation is performed. It contains all the 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 operation is called a sequence and holds the individual sample information, references to the appropriate methods and automatic recalibration specifications.

System Configuration

The configuration of the instrument system is done through the configuration editor program. It allows users to define their instruments,GP-IB addresses, IP LAN addresses, the directories for data, sequences and methods, and the color definition for the ChemStation software.

Data Model

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

The ChemStation provides, commands and functions to construct, expand, extract and, where it does not alter primary data, edit registers.
Registers hold information about their contents in register headers. The registers are further subdivided into one or more objects. Typically an object holds data that describes an analytical measurement, such as a chromatography signal. Each of these objects have their own header with information about the analytical measurement such as the data file name, injection date and time, sample name, and tables. Tables are used to hold different types of data as one block of information. For example, the quantification process in a calibrated method constructs a quantification table that contains peak numbers, compound names, compound amounts and retention times.

Like other parts of the registers, tables may be user-defined and have the functionality of database tables with the additional benefit of being directly associated with the base piece of analytical information from which they were derived.

Each register may hold information for different purposes. As well as analytical data, the register data model is used for holding configuration information and analytical methods. They may be saved as files on non-volatile storage and reloaded into the ChemStation memory, printed and plotted to the screen or a hardcopy device. Their binary format means they are not editable outside the ChemStation and each data item may also be protected by assigning access attributes to it when it is created.

Their design fits extremely well to modern database technology enabling systems to be developed to map analytical results or data directly to a relational database system.

Software User Interface

The Agilent ChemStation user interface is designed into Views that group software functionality according to typical analytical tasks. 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 re-evaluating data that has been acquired.
- The *Report Layout* view for designing specific report layouts.

Additional views are present if additional data evaluation modules have been installed or for certain instrument configurations that support instrument diagnostics and verification procedures.

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, LC/MSD 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 appropriate modules are installed. Each of these separate data analysis modes are supported with a mode-specific toolset.

The *Report Layout* view allows the user to graphically define the layout of a specific report style in a graphical object orientated fashion. It too 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 or oven temperature profiles and gas flow or pressure for gas chromato-graphy, may be recorded and stored with each data file if the configured instrument supports this capability. 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 parame-

ters, 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.

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:

- Single or multi-signal 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.
- Export of time/intensity digitized points to the Microsoft Windows clipboard.

Data Analysis - Integration

The Agilent ChemStation includes two integration algorithms. The traditional integration algorithm was included in earlier versions of the Agilent ChemStation and is also included in most other Agilent analytical data evaluation software. The enhanced integration algorithm is aimed at improved ruggedness, reliability and ease-of-use.

In this software revision, it is still valid to use the traditional algorithm for exisiting validated methods. We recommend using the newer algorithm when modifying methods or creating new ones.

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.
- 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 new integration algorithm has improvements for

- peak baseline allocation on wandering baseline chromatograms or electropherograms,
- 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,
- user-selectable exponential or straight tangent skim mode which is also displayed on the integrated chromatogram, and
- ease-of-use — the enhanced integrator algorithm has a new user interface based on tool bars and automatically focusing on key information.

Data Analysis - Quantification

The Agilent 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. Individual calibration point weighting may be specified as
- equal — all calibration points are considered of equal importance in drawing the calibration curve.
- based on the number of calibrations — the significance of a point on the calibration curve depends on the number of times it has been recalibrated.
- linear fit — the importance of a point is the reciprocal of the compound amount of each calibration level.

- quadratic fit — the importance of a point is the square of the reciprocal of the compound amount of each calibration level.

Calibration curves are constructed for each compound by using the calibration levels to define the curve. The algorithm used to generate the calibration curve may be selected as
- piecewise — a point-to-point extrapolation,
- linear — a linear regression fit of the data points,
- quadratic — a quadratic fit of the data points,
- cubic — a cubic fit of the data points,
- exponential — an exponential fit of the data points,
- logarithmic — a natural logarithmic fit of the data points,
- power — a power fit of the data points, and
- average response/amount.

The origin of the calibration curve may be specified as
- ignored — the origin (0,0) is not used in the curve calculations,
- included — the origin is used as one of the calibration points,
- forced — the curve is forced through the origin, and
- connected — the linear segment is constructed between the origin and lowest calibration point on the curve.

Compound identification may be refined by defining individual retention time windows' parameter limits and qualifier peaks.

Qualifier peaks are usually the same compound detected on a different signal with a predictable response ratio. They are used as a further check on peak identification rather than just relying on retention times.

Each calibrated compound may have individual absolute limits for the amount, peak area, peak height, symmetry, efficiency in plates, resolution and k'. Results lying outside any defined limits are indicated on the appropriate analysis report. They may be used in conjunction with the control samples that can be defined as part of the automation setup, to verify the performance of the system running automatically. The ChemStation can calibrate methods with up to 1000 peaks and 2000 calibration points. This means, for example, with 1000 calibrated peaks only two calibration levels may be defined for each peak. With fewer peaks more levels may be defined in proportion to these limits (for example, 100 compounds could have 20 levels each).

The group calibration capability allows the user to group calibrated peaks into a named group and report quantitative results for both the individual group members and the group itself.

The Agilent ChemStation for CE allows calibration tables to be built based on peak area, height or corrected peak area. The additional corrected peak area option automatically corrects for varying elution speed, inherent in the capillary electrophoresis technique.

Data Analysis - Standard Reporting

A standard set of user definable report styles for sample reporting are selectable from the report specification screen. Every report type contains standard information groups and a series of optional information groups.

The standard groups include
- A header with the originating data file and sample name.
- A footer with the instrument name, operator name, print time and page number in 'page x of y' format.
- A sample information block that includes sample name, vial number, method and sequence information, operator and instrument name and sample information text.
- A quantification results table containing retention times and the integration or quantification table depending on the calculation scheme chosen. This table can be formatted either by retention time or by signal.

Users may select from a series of optional information groups by specifying a particular style for the analytical report. These groups include
- A front page that can include user-defined text.
- Repetition of the sample information block on every page.
- Instrument conditions.
- The analytical column or capillary specification for LC, LC/MSD and CE systems.
- The run logbook.
- The chromatogram with annotation options that include selection from peak retention times, compound names, tick marks, baselines and axes. The user may also select the annotation fonts, graphical orientation, size and whether the graphics are overlaid or separated. If the ChemStation is connected to instruments that can record instrument parameters as a signal, such as temperature, flow and pressure, the user may also select to include these graphics in the report.
- Calibration table and calibration graphics.

Reports may be output to the screen, printer or to file in any combination.

If the screen is selected as one of the report destinations, the report together with graphics, will be shown in a separate window from which users can then select to print the report to the printer.

For file output ASCII, comma-separated values (CSV) and data interchange (DIF) formats and Microsoft excel (XLS) formats may be selected for text and numeric information. Windows metafile (WMF) format is used for graphics. CSV, DIF and XLS are standards used commonly by spreadsheet programs. Additionally, resutls vcan be generated in hypertext markup language (.HTML) for viewing in web browsers.

Users may set up the Windows printing devices from within the ChemStation software, using the Printer Setup function.

The Agilent ChemStation for CE has an additional mobility report that uses the voltage signal and the electropherogram to compensate for the velocity of the compounds migrating through the detector cell.

Data Analysis - Specialized Reporting

Advanced reporting capabilities are also included in the ChemStation for users who require a more specialized set of reports. These include statistics on separation quality, reports that include trend analyses 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 includes
- retention time,
- k',
- peak area,
- peak height,
- symmetry,
- true peak width at half height,
- efficiency in plates,
- 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 a signal-to-noise ratio 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.

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, LC/MSD 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,
- statistics on calibration samples,
- statistics on unknown samples, and
- 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.

The statistical reports may be selected as standard or extended styles. The *Standard Style* is text-based and includes the mean, standard deviation (SD), relative standard deviation (RSD) and standard error for the following parameters tabulated by compound:
• retention time,
• area,
• height,
• peak width, and
• peak symmetry.

The *Extended Style* includes graphical trend analyses based on a selection of parameters for statistical evaluation. The parameters that can be selected include
• retention time,
• area,
• height,
• amount,
• peak width at half height, by the sigma, tangent and tailing methods,
• peak symmetry,
• tailing factor,
• k',
• theoretical plates by the peak width at half height, sigma, tangent and statistic methods ,
• resolution by the peak width at half height, sigma, tangent and statistic methods,
• selectivity,
• skew, and
• excess.

Technique specific parameters for liquid chromatography include
• peak purity evaluation factors (with the diode-array spectral evaluation module only), and
• spectral library comparison factor (with the diode-array spectral evaluation module only).

The report includes a separate graphical trend analysis for each selected parameter.

Sequence summary reports may be output to the printer, to file or both. The user may select to either print or suppress individual analysis reports together with the sequence summary.

Customized Reports

A customized reporting design view is included in the Chem-Station 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 sections and graphically adjust the relative position, size and orientation of each defined element. The individual sections may be added, deleted, re-ordered 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, a printer or a file. Reports to the screen include graphics.

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). 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 Agilent ChemStore product.

LC and CE ChemStations

Methods, data files, spectral libraries and sequences from the previous versions of the LC ChemStations, revisions A.01.00 to A.08.04 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 'Customization' section) to convert chromatographic data into the new LC ChemStation format.

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

Customization

The ChemStation can be customized using a powerful command set . These commands may be grouped to automatically execute 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.

As well as using simple scalar or string variables, data may be stored as tables. Users have access to all the ChemStation data through standard data structures and may even define their own tables for specialized applications.

The key chromatogram object manipulation commands include addition, subtraction, multiplication, ratio, comparison and calculation of regression coefficients between two objects. Single chromatogram objects may have the following operations applied to them: derivatization, exponential function, natural log function, reciprocal function, cubic spline function and a time or intensity shift.

The documentation contains complete descriptions of the ChemStation data structures, variables and a guide to customization with practical examples.

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 multi-method sequences for all the autosamplers noted in the instrument control sections that follow.

The sequence parameter set may be defined to use automatically generated or user-defined file names. 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 users may also specify an injection volume for LC applications. 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.
- 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 are analyzed between two calibration sets. 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.

Batch Review is an additional mode of data analysis that provides automation by allowing a first-pass review of a batch of samples quickly and easily. The batch consists of all or a selection of runs from a sequence. You can check the calibration accuracy and the individual integrations before approving the results. All chromatogram-specific modified integration parameters can be saved for data traceability. Once data is accepted the entire batch can be reprocessed to generate reports with one keystroke.

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 help users validate and specify methods, verify system performance and ensure the traceability, originality and quality of the data.

Development Process

The Agilent 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, registered to the ISO 9001 quality standard, is documented together with on-site revalidation protocols in the validation binder *Agilent ChemStation for LC and LC/MSD*.

Method Specification and Use

- Global methods — the complete instrument and data analysis specification is stored as one entity. 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 add a reason for the change 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 'Reporting' sections) provide detailed analysis of the separation quality.
- The ChemStation may be configured for restricted access through 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 to 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 section on specialized reporting, pages 12-13) 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 software verification kit, that is part of the standard software, automatically checks for the correct operation of the data evaluation parts of the software by comparing results generated when a test is executed against pre-recorded known values. The verification test allows users to define their own data files and methods to be the basis of the test.

Data Traceability, Originality and Quality

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

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

- Methods saved with the data file records 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' style). The user may select the level of detail in each report ranging from simple summary reports to complete system details (see reporting section).

- 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.

- Data security is achieved in the PC environment through password protected PCs, software locks built into both Microsoft operating systems and secure (password-protected) networks.

Instrument Control

The instrument control capability of the Agilent ChemStations may be expanded through the purchase of additional instrument modules to allow multiple instrument, mixed technique configurations.

The instrument control capabilities are documented in the following sections, each relating to a specific technique.

Agilent ChemStation for GC

Instrument Control and Data Acquisition with the Agilent ChemStation for GC (G2070AA) and the Additional GC Instrument Module (G2071AA)

The Agilent ChemStation for GC combines instrument control, data acquisition, and data analysis software for the Agilent 6890, 6850, 5890 Series II, 4890 A, and 4890 D gas chromatographs and the Agilent 35900E A/D converter.

The Agilent ChemStation is interfaced to the GC via GP-IB, RS-232, or LAN and collects full range digital data from detectors. Depending on the detector type, data can be acquired at rates up to 50 Hz from the Agilent 5890 and Agilent 4890 Series and up to 200 Hz from the Agilent 6890 and Agilent 6850 Series .

When interfaced to an Agilent 5890 Series II gas chromatograph, the Agilent ChemStation can control all GC parameters for heated zones, oven temperatures, detectors, inlets, cryogenic cooling, signals, electronic pressure control, cool on-column temperature programming, and CryoBlast.

The Agilent ChemStation can control, through a timetable, the four 120V valves and four relays of the Agilent 19405A or the eight relays of the Agilent 19405B event control module interfaced to the Agilent 5890 Series GC through INET.

When interfaced to an Agilent 6890 or Agilent 6850 Series gas chromatograph, the Agilent ChemStation can control all GC parameters for heated zones, oven temperatures, detectors, inlets, cryogenic cooling, signals, electronic pressure control, cool on-column temperature programming, and CryoFocus.

In addition the following features have been added:
- Graphical user interface for easy access to all method areas for all Agilent GCs
- Table driven system scheduler which permits clock time programming for all Agilent GC so Quick Method which allows the new Agilent Series 6890 and Agilent 6850 GC user to enter a limited number of

setpoints from which the Agilent ChemStation will create a method
- Method resolution in the Agilent ChemStation (Agilent 6890 and Agilent 6850 Series only) will verify and alert the operator of potential problems if a method was created on a different GC system or if the configuration has changed
- Column calibration capability

The Agilent ChemStation can display graphically the oven temperature, inlet temperature, inlet pressure, auxiliary channel pressure and column flow programs. The Agilent ChemStation also can control, through a timetable, a maximum of eight valves or relays.

Sampling

The Agilent 7673 and Agilent 7683 Series automatic samplers allow for complete automation of sample introduction in single front, single rear or dual-injector configurations.

Dual-injector configurations (Agilent 6890 and Agilent 5890 Series only) allow individual or synchronous injections. Each automatic sampler allows a 3-vial turret, 8-vial turret, or 100-vial access if a sample tray is fitted. (6890 and 5890 Series only). The Agilent ChemStation allows random sample access and priority sample injection.

The following autosampler parameters may be controlled:

- number of syringe pumps
- number of syringe washes
- the injection volume
- the bottle number for each injection
- a viscosity delay
- on-column injection setup
- syringe size
- depth of needle penetration
- multiple injections per run in cooperation with PTV for large volume injection
- for the Agilent 7683 Series , plunger speed may be controlled from a maximum of 100 µl/sec to a minimum of 5 µl/sec when using a 10 µl syringe for large volume injections o a 100 µl syringe can be used with the Agilent 7683 Series for large volume injections

The Agilent ChemStation allows the user to optionally display a Sampling Diagram window containing a graphical display of the one hundred vial tray, indicating which samples have already run, which sample is currently running, and which samples will be run.

The G1926A bar code reader attachment is supported in the 100-vial tray configuration of the autosampler. The bar code reader can be used to help build automation sequences and verify that the identity of the injected sample matches the name in the sequence table at injection time.

The Agilent ChemStation can acquire a third and fourth signal from external detectors in a single run by adding the 35900E A/D converter.

Agilent ChemStation Companion

The Agilent ChemStation Companion is an optional add-on to the GC ChemStation that provides the user with another, simplified view. In the Companion View, the user is limited to selecting pre-programmed samples, methods, vial numbers, and run control. Users cannot modify or create any methods or run any methods or samples not assigned to them by their lab manager.

To load the Agilent ChemStation Companion, run "Setup.exe" from the Agilent ChemStation Software CD-ROM in the "Companion" directory.

Retention Time Locking Software

Retention Time Locking (RTL) software, product number G2080AA, is an add-on to the G2070AA GC ChemStation. G2080AA will only install on the GC ChemStation. There is a G2081AA RTL Pesticide Library available.

Retention Time Locking is an interactive software tool kit that includes a calculator and empty

table. The calculator uses the RTL equation to create a calibrated curve of retention time verses pressure. This curve is used to calculate a new pressure to give the desired retention time. RTL is the ability to match the retention times of one system to those in another chromatographic system, with the same nominal column.

It is much easier to identify peaks and validatemethods if there is no variation in the retention time of each analyte. The empty table is designed to allow the user to create a library for compounds of interest. The RTL Pesticide Library, product number G2081AA includes the retention times for 567 pesticides and suspected endocrine disrupters. To use this library, the RTL software product G2080AA must be installed. Peak identification is performed by comparing the retention time of the unknown peak with that of a standard included in the library table.
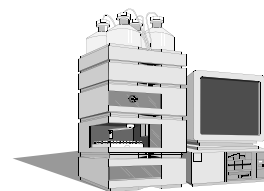
Agilent ChemStation for LC Systems

Instrument Control and Data Acquisition with the Agilent ChemStation for LC Systems (G2170AA) and the Additional LC Instrument Module (G2171AA).

The Agilent ChemStation for LC and additional LC instrument module controls and acquires data from the Agilent 1100 Series modules and systems for LC, the HP 1090 Series liquid chromatography systems with either the filter photometric detector (FPD) or built-in diode array detector (DAD), the stand-alone HP 1040 diode array detector (DAD), the HP 1050 Series of liquid chromatography modules, the HP 1046A fluorescence detector (FLD), the HP 1049A electrochemical detector (ECD) and the Agilent 35900E dual channel interfaces. All the sampling, pumping and detector options of the Agilent 1100 Series modules and systems for LC, the HP 1090 Series and HP 1050 Series liquid chromatographs can be controlled.

Sampling Systems

The injection systems may be manual or automated with an autosampler or well-plate autosampler. All automatic injectors may be programmed for different injection volumes, the speed of injection and the injector wash procedure. The user may also specify a complete injector program for sample dilution, standard addition or sample derivatization. The commands available for the injec-

tor program include draw, eject, mix, wait, inject, sampler valve and column switch control. These can be defined in conjunction with the sample, a vial/well-plate offset from the sample, a numbered vial/well, waste and air.

The Agilent bar code reader attachment is supported in the 100-vial tray configuration of the HP 1050 autosampler. The bar code reader can be used to help build automation sequences and verify the identity of the injected sample matches the name in the sequence table at injection time. In LC configurations it can also be used to mix liquid samples as a step in an injection program.

The following Agilent 1100 injection systems are supported:
- Standard autosampler (G1313A)
- Thermostatted standard autosampler (G1327A)
- Well-plate autosampler (G1367A)
- Thermostatted well-plate autosampler (G1386A)
- Thermostatted micro auto-sampler (G1387A)
- Micro well-plate auto-sampler (G1377A)
- Thermostatted micro well-plate autosampler (G1378A)
- Agilent 220 micro plate sampler (G2250A/G2251A). Dedicated add-on software is needed for these two modules.
- Preparative autosampler (G2260A)
- Thermostatted preparative autosampler (G2261A)
- Agilent fraction collector (G1364A)

Solvent Delivery Systems

All the solvent delivery systems have a set of initial parameters, including pressure limits, initial flow and composition, that are complemented by a time-table for programming changes in flow, composition and pressure limits. These parameters can be viewed graphically. Users can define a post-run time for column equilibration.

The following Agilent 1100 solvent delivery systems are supported:
- Isocratic pump (G1310A)
- Binary pump (G1312A)
- Quaternary pump (G1354A)
- Preparative pump (G1361A/G1391A)
- Capillary pump (G1382A)

Column Compartments

The Agilent 1100 Series thermostatted column compartment can be set between 10 °C below ambient and 80 °C. The temperature is programmable during the run through a timetable. The HP 1090 column oven temperature can be set to a constant temperature (20 °C above ambient to 100 °C without external cooling). The HP 1050 heated column compartment can be set to a constant temperature (5 °C above ambient to 85 °C). Column switching valves are programmable from the software.

Detectors

Spectral data from all diode-array detectors may be acquired in a peak-controlled or full acquisition mode.

The ChemStation software can simultaneously acquire five chromatographic and reference signals each with an independent bandwidth from the *Agilent 1100 Series diode array detector* and the *HP 1050 diode array detector*.

The system can simultaneously acquire up to a total of eight chromatographic and reference signals from the *HP 1090 or HP 1040 diode-array detector (DAD)*, each with an independent bandwidth.

The detectors have a graphical test for signal intensity and wavelength calibration. All DADs may have the initial parameters changed during a run by a time-based program. Users can program wavelength and spectral acquisition mode changes in the time table.

Initial parameters that may be set for the DADs include signal wavelengths and reference wavelengths, spectral acquisition mode, signal sampling rate and autobalance.

The stand-alone *HP 1040 diode-array detector* is implemented identically to the diode-array detector built into the HP 1090 Series liquid chromatographs. Configurations with a diode-array detector have a spectral monitor window to show the spectra being acquired both during and between analyses. Users may interactively change the absorbance and wavelength of this display.

The *HP 1090 filter photometric detector (FPD)* may be programmed with parameters to set the lamp current, response time and the filter. The filter may be changed during an analysis through events in the detector's time table. The ChemStation includes a diagnosis screen for testing the reference and sample photo diode light paths in the detector. The FPD is interfaced to the ChemStation through the digital GP-IB interface for control and through a dual channel A/D interface for the data acquisition (see below).

The Agilent 1100 Series variable wavelength detector (VWD) may be programmed with a single detection wavelength. Data acquisition rates may be programmed for peak widths from <0.12 up to 8.00 seconds. The VWD can be programmed with a timetable to change the wavelength and perform wavelength scans during the course of an analysis.

The Agilent 1100 Series multiple wavelength detector (MWD) can simultaneously acquire up to five chromatographic signals each with independent reference wavelengths and bandwidths. The signal acquisition rate may be set for peak widths between 0.1 and 16 seconds. During the course of an analysis the MWD can be programmed with a timetable to change wavelengths, bandwidths and peak-widths for all five wavelengths.

The optical unit temperature of the Agilent 1100 Series Refractive Index Detector (RID) can be set between 20 and 55ºC. The signal acquisition rate may be adjusted for peak widths from <0.12 up to 8 seconds. During the course of an analysis the RID can be programmed with a timetable to change Polarity and Peakwidth of the acquired chromatographic signal. For diagnostic and troubleshooting purposes, it is possible to store Diode Signal 1, Diode Signal 2, Optical Unit Temperature, Polarity and the Balance Signal in addition to the chromatographic signal.

The *HP 1050 Series variable wavelength detector (VWD)* may be programmed with a detection wavelength and data acquisition rates (from 0.013 to 1.60 minutes peak width). The user may program wavelength changes to occur during an analysis.

The *HP 1050 Series multiple wavelength detector (MWD)* may be programmed with up to three detection signal wavelengths, each with independent reference wavelengths and bandwidths. Wavelength changes may be programmed to occur during an analysis. The signal acquisition rate may be set for peak widths between 0.01 and 0.85 minutes.

The excitation and emission wavelengths of the *HP 1046A fluorescence detector (FLD)* can be set from 190 to 800 nm, in steps of 1 nm. Gain, response time, gate, delay and lamp frequency may also be set. Gain and changes in the emission and excitation wavelength may be time-programmed. The excitation and emission wavelengths may be optimized by analyzing scans. The range and speed may be specified for each scan during the optimization process.

The scans taken during an analysis are stored in a ChemStation spectral file format that allows them to be displayed and used in a spectral library.

The Agilent 1100 Series fluorescence detector (FLD) may be programmed for single wavelength or simultaneous multiple wavelength detection and spectra aquisition. Up to four signals at different exitation or different emission wavelengths may be obained. Within a timetable initial exitation or emission wavelengths, response time, PMT Gain and baseline behaviour as well as spectral parameters may be changed. Exitation or emission spectra can be watched online and stored and analyzed as described for DAD spectra. For a single compound trapped in the flow cell, complete information on exitation and emission spectra is available in a single task with the fluorescence scan and can be watched as an iso-plot or as 3D-graphics.

The *HP 1049A electrochemical detector (ECD)* may be used in amperometry, pretreatment, sweep, pulse and differential mode. The voltage potential and limits may be defined between -2.0 V and +2.0 V in steps of 0.001 V. A voltage potential increment between analyses may be defined from -2.0 V to +2.0 V in steps of 0.001 V and the number of repeat analyses at a given increment may be set. Drift limits for

the detector not ready condition may be specified from 0.1 nA to 500 nA in steps of 0.1 nA. The user may also specify auto-zero control, based on the prepare or stop signal or a user defined drift value, full scale detector output at 0.05 µA, 0.5 µA or 500 µA, the signal polarity and the temperature of the solvent thermostat (20 to 60 °C).

The *Agilent 35900E dual channel interface* allow the system to acquire data from detectors that are not interfaced for data acquisition through the GP-IB system or LAN, such as the FPD, the HP 1047A refractive index detector or a third party detector. One or two analog signals per instrument may be configured; if only one is used the other is available for use with another instrument. Data rates up to 100 Hz per signal may be defined. The user may also define the units for acquisition and their relationship to the voltage signal (units/volt).

The Agilent 35900E interfaces offer external event control through digital TTL (transistor-transistor logic) signals, each of which are given specific state (high and low) names, that may be time-programmed before, during and after an analysis. The Agilent 35900E can be configured for up to eight signals for each independent channel.

Agilent 1100 Series Instrument Verification

The Agilent ChemStation for LC includes an instrument operational qualification and performance verification (OQ/PV) view in which users of the Agilent 1100 Series of modules and systems may select a series of semi or fully-automated tests to test the operational suitability of the LC instrument.

Instrument verification includes one or more of the following tests which can be selected by the user:

☑ System tests
- chromatographic perfor mance tests
- carry over determination
- system linearity

☑ Pumps
- leak tests
- gradient performance test (binary and quaternary pumps only)

☑ Automatic and manual injectors
- injection precision

☑ Heated column compartments
- temperature accuracy
- temperature stability

☑ Diode-array and variable wavelength detectors
- wavelength calibration
- noise and drift

Users may select sets of appropriate tests from the list of available tests. The user selected test set may be saved under specific names for easy repetition of the test set at a later date.

Each individual test has both warning and control limits. If control limits are exceeded during the execution of a test, the execution of the entire test suite will be halted. Exceeded warning limits are noted on the test report. Both warning and control limits may be defined by the user.

Once the test suite has been completed the results associated with that suite may be reported and saved with the test suite.

The instrument verification test report includes:

☑ Tested instrument's configuration and the PC specification
☑ Scheduled tests
☑ Test results comprising
  • overall test result,
  • test status in either a short (pass/fail) or verbose mode that additionally lists each test limits and the measured value,
  • result graphics, and
  • normal reports of method runs that are run as part of the test.

Test sets saved with results may be reloaded at a later date for re-running or re-reporting.

The *Operational Qualification and Performance Verification Manual*, supplied in paper and electronic format, provides descriptions of the purpose, scope, parts, materials, procedures and theory of each test. The electronic format allows users to readily incorporate this information into their own laboratory standard operating procedures.

Agilent 1100 Series Diagnostics

The Agilent 1100 Series of systems and modules for LC have an additional ChemStation diagnosis view.

The diagnosis view is designed to help users identify instrument malfunctions starting from a particular symptom. A failure of a particular instrument verification test will automatically identify the appropriate symptom for the user or the user may select the symptom interactively.

One or more possible causes is listed for each symptom. Each possible cause is associated with a series of diagnostic measurements, with limits, and a series of diagnostic tests. Users observe the measurements and carry out the tests in order to confirm or dismiss the possible cause of the instrument malfunction.

Once identified, the cause of the instrument malfunction may be repaired by using the repair procedures given on the Agilent 1100

Series Maintenance and Repair CD-ROM. The repair procedures include parts and materials breakdowns and clear animated step-by-step graphics or video with a sound track for each repair procedure. The procedures are called directly from the Agilent ChemStation diagnostics view.

Agilent ChemStation for LC/MSD Systems
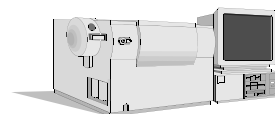
Instrument Control, Data Acquisition, and Data Evaluation with the Agilent Chem-Station for LC/MSD Systems (G2710AA) and the LC/MSD ChemStation Add-on Module (G2715AA)

The Agilent ChemStation for LC/MSD systems (G2710AA) and the LC/MSD ChemStation Add-on module (G2715AA) provide control, data acquisition, and data evaluation capabilities for Agilent 1100 Series LC/MSD systems. G2710AA includes the G2170AA LC Chem-Station and G2180AA diode-array detector (DAD) spectral evaluation module, as well as the G2715AA LC/MSD Add-on module. Together, these software components provide integrated control with a common graphical user interface for all of the Agilent 1100 Series LC modules and systems, including the Agilent 1100 Series DAD as well as the Agilent 1100 Series LC/MSD. In addition to the Agilent 1100 Series family of modules and systems, the HP 1090 Series II liquid chromatography system, as well as the Agilent 35900E A/D interface can be controlled by G2710AA as part of LC/MSD systems. The Agilent ChemStation for LC/MSD system supports a single Agilent 1100 Series LC/MSD system.

LC/MSD System Control

The software provides digital control of the LC/MSD API ion source and ion optics, dynamic ramping of ion optics element voltages, and control for spraying and drying gases. Method-specific LC/MSD parameters include spectral acquisition mode (scan/SIM), signal sampling rate, LC/MSD tune file, ionization mode (APCI, APPI or API-ES mode) and polarity (positive or negative ion detection). Within a LC/MSD method, LC/MSD-timed events include mass range, SIM ion groups, mass analyzer stepsize, fragmentor voltage, electron multiplier gain, MS on/off, and API ion source parameters. Fragmen-tor voltage settings may be dynamically ramped within a scan to optimize response for various m/z values. LC/MSD operating parameters such as fragmentor voltage, drying gas temperature, and EMV gain can be acquired and saved with a data file. These instrument parameters can be displayed and plotted as a record of the exact values associated with the acquired data.

In addition to the standard ChemStation automation capabilities for single run methods and mutiple method sequences, an FIA (Flow Injection Analysis) Series automation mode is available through software selection. In this mode, which requires the Agilent 1100 Series LC autosampler, the Agilent 1100 Series LC/MSD system can be programmed to make multiple injections from a single or multiple sample vials, storing all data in a single datafile. Up to two LC/MSD method parameters can be programmatically varied with each injection.

LC/MSD Tuning

The Agilent ChemStation for LC/MSD systems includes a LC/MSD tune view in which users of the Agilent 1100 Series LC/MSD may select to either automatically, or manually tune the instrument. The Agilent 1100 Series LC/MSD integrated calibrant delivery system is software-controlled, and together with the software autotune provides fully automated tuning of the Agilent 1100 Series LC/MSD for API-electrospray (API-ES), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI) modes of operation. An extensive set of manual tune capabilities is also provided for users who wish to manually tune the Agilent 1100 Series LC/MSD.

Agilent 1100 Series LC/MSD Instrument Verification

Computer-aided operational qualification and performance verification (OQ/PV) tests and procedures can be used to verify that system performance is acceptable on an ongoing basis. Early maintenance feedback (EMF) tracks the status of system maintenance items and provides notification when a preventive maintenance procedure is due. On-line diagnostics enable system troubleshooting using integrated tests. System logbooks provide date- and time-stamped records of runs, errors, and maintenance events.

The user can select from any of the following tests:

☑ Injector Precision test
- This test verifies that the injector precision is within the acceptance criteria.

☑ Detector Linearity
- This is to verify that the detector shows a linear response with an increasing sample concentration.

☑ Carry-over test
- This test is to verify that the sample carry-over (sample contamination from a previous injection) between injections is with in the acceptance criteria.

☑ Injector Linearity test
- This test verifies that the injection volume linearity is within the acceptance criteria.

Diagnostics/Early Maintenance Feedback

The Agilent 1100 Series LC/MSD software extends the diagnosis view of the existing LC ChemStation to include tests for the Agilent 1100 Series LC/MSD. The diagnosis view is designed to help users identify instrument malfunctions starting from a particular symptom. Maintenance and repair procedures for the Agilent 1100 Series LC/MSD can be called directly within the diagnosis view from the Agilent 1100 Series LC/MSD Maintenance and Repair CD-ROM. The procedures include parts and materials breakdowns and clear animated step-by-step graphics and multimedia clips for each repair procedure.

Early Maintenance Feedback (EMF) automatically notifies the user when maintenance is required for key system components such as rough pumps, calibrant delivery system, spray chamber, and electron multiplier.

LC/MS Technical Primer

The Agilent ChemStation for LC/MSD includes an on-line multimedia LC/MS technical primer. The primer includes information to assist users of the Agilent 1100 Series LC/MSD system with a variety of LC/MS technical topics, including API-LC/MS theory, method development and solution chemistry for API-LC/MS, and CID (collision induced dissociation).

LC/MSD Data Evaluation

The LC/MSD ChemStation includes all of the data evaluation capabilities of the Agilent 3D Chem-Station for LC, including data evaluation for UV-visible spectra acquired from a supported diode array detector (DAD). In addition, the LC/MSD Chem-Station includes capabilities for evaluation of mass spectral data acquired from the Agilent 1100 Series LC/MSD module.

Both UV-visible and LC/MSD data can be viewed, compared, and printed. Chromatograms from the separate detectors may be simultaneously displayed, aligned, and resized to correlate peaks from one chromatogram to the other. Mass spectra and UV-visible spectra can be simultaneously reviewed using a common spectral toolset. Reports can include either UV-visible or mass spectral data, or both.

Interactive Data Processing

The data from the mass selective detector may be displayed in a number of ways. The total ion chromatogram (TIC) is the summation of all mass signals (m/z values) over the entire acquired data range. An extracted ion chromatogram (EIC) displays the signals of individual ions (m/z values) or a range of m/z values.

The mass selective detector signals (TIC and /or EICs) may be displayed along with those from other LC detectors. The software permits peak alignment for chromatograms from different detectors connected in series. Full, comparative mass and UV-visible spectra manipulation are available including selection of spectra by:

- individual spectrum,
- peak apex spectrum,
- average spectrum over a graphically defined retention time range
- range of spectra, and
- all spectra over a peak.

The user may also select how the spectra are processed when they are displayed. The available options include:

- background subtracted spectra,
- limiting the m/z range,
- smoothing,
- normalization, and
- continuous curves or histogram mode.

Quantification

All of the standard ChemStation quantification capabilities are available for use with mass spectral data. TIC or EIC signals can be used for quantification. For target compound analysis, retention time windows, quantification ion signals, and qualifier ion signals/ratios can be defined on a per-compound basis.

Peak Purity

The LC/MSD ChemStation includes all the UV-visible peak purity data evaluation capabilities of the Agilent diode array detector (DAD) spectral evaluation module. Capability for peak purity determination using LC/MSD mass spectral data is also included. Peak purity may be determined interactively on either a peak by peak basis, for all the peaks from a certain data file, or automatically at the end of an analysis as part of the method.

The user can select to interactively evaluate peak purity for data sets that include both DAD spectral data and LC/MSD spectral data in either a single or dual mode. In single mode, the software configures the purity user interface for evaluation of data from either one of the two data types at a time. The user can toggle between the data types if desired. The dual mode user interface permits simultaneous evaluation of spectral purity using both DAD and LC/MSD data.

In interactive operation, the LC/MSD peak purity function examines the most significant ions across a user-selected chromatographic peak to determine if more than one compound is present. The software automatically overlays extracted ion chromatograms for the selected peak, with each extracted ion chromatogram displayed in a separate color. A table of the number of components

located and the two most significant ions used to resolve each component is displayed. The next/previous peak or the next/previous impure peak can be selected by simple mouse actions. A purity report that includes peak purity assessment for all peaks in a chromatogram can also be specified and displayed/printed.

Iso-abundance Plot and Three Dimensional Plot

In addition to iso-absorbance and three dimensional plots for UV-visible spectral data, the LC/MSD ChemStation also provides equivalent capabilities for mass spectral data.

The MS iso-abundance plot displays acquired mass spectra as a color-contoured map of m/z against retention time together with areas for display of m/z signals and mass spectra defined by the position of cross-hairs on the iso-abundance plot. In the iso-abundance plot, a color scale is used to represent signal intensity. Users can define the contour color schemes and retention time and m/z ranges for the display.

Acquired mass spectra can also be displayed as a three dimensional plot of m/z against retention time and abundance. The display can be graphically adjusted by the user in the time, m/z, and intensity domains. The resolution of the plot is selectable, and the orientation of the plot can be adjusted graphically. The plot may be printed, and the color scheme adjusted.
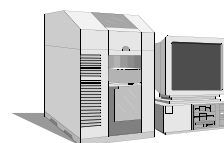
Instrument Control, Data Acquisition and Technique-Specific Data Evaluation with the Agilent ChemStation for Capillary Electrophoresis (G1601A) and the Additional CE Instrument Module (G2172AA)

The Agilent ChemStation for CE and additional CE instrument module controls and acquires data from the Agilent capillary elec-trophoresis instrument as well as providing technique-specific data processing capabilities for the configured instrument.

The main parts of the instrument are the 48-vial autosampler carousel, a pressure system for moving electrolyte, buffer and injecting sample in pressure injec-tion mode, a capillary cassette, a high voltage power source and a diode-array detector identical to the HP 1050 DAD.

The CE instrument status is shown through a technical graphical representation on the ChemStation screen. This graphi-cal user interface (GUI) allows direct access to key instrument parameters through the symbolic representation of the instrument on the screen. Users may, alterna-tively, choose to set the instru-ment parameters through the menu or the CE method screen that presents the instrument con-trol categories as a list. Once a CE method has been defined, it's integrity may be checked through a method run simulation capability.

The CE method categories are:

Capillary Vials and Temperature Settings Home Values

The depth to which the capillary penetrates the vials may be set between 4 and 18 mm. The cas-sette temperature may be set between 4 and 60 °C and the vial numbers of the default inlet and outlet buffer vials referenced by name in the system's time table, may be defined.

Replenishing, Pre- and Postconditioning Setup

Replenishment, preconditioning and postconditioning may be indi-vidually omitted, done at the same time or done sequentially. The replenishment time table, used to replace buffer solutions in the vials, may include replenish, fill, empty and wait steps applied to the default inlet and outlet vials or any vial of the method developer's choice.

The preconditioning time table, executed before an analysis pri-marily to condition the capillary, may include capillary flushing, pressure, voltage, current and power settings, wait steps and the selection of user defined vials into the inlet and outlet positions. The postconditioning time table, executed at the end of an analysis, has identical capabilities to the preconditioning time table.

Parameters for Injection

Sample injection may be done by voltage, current, pressure, through the instrument timetable or omitted.

The Agilent CE instrument may also be used in isocratic capillary electrochromatography (CEC) mode in which a packed capillary is used under 2-12 bar pressure.

Electrical Parameters for Separation

The electrical parameters may be switched on and off through the software. The polarity of the sys-tem may be set positive or nega-tive. The following electrical para-meters may also be set:
• power, between 0 and 6 W or to the instrument limit,
• voltage, between 0 and 30 kV or to the instrument limit,
• current, between 0 and 300 μA or to the instrument limit, and
• lower current alarm limit, between 0 and 300 μA or to the instrument limit

Analysis Stop time, Post time, Raw Data and Timetable Settings

The software may be set up to continually store, in addition to the signal and spectra defined by the detector setup, the following instrument parameters:
• voltage
• current
• power
• pressure
• cassette temperature

The stop time defines the length of the analysis and may be set between 0.20 and 99,999 minutes or set to unlimited. A post analysis time, when the system is held in a not-ready state, may be defined between 0 and 99,999 minutes.

The instrument may also be programmed to execute a timetable during each analysis. The timetable may change separation and injection parameters including the polarity, voltage, current, power, pressure and temperature as well as the alarm current limit and inlet and outlet vials.

Detector Setup

Spectral data from the diode array detector may be acquired in a peak-controlled, full acquisition mode or not at all. The ChemStation can simultaneously acquire up to five chromatographic signals from the DAD each with an independent reference wavelength and bandwidth. The detector has a graphical test for signal intensity and wavelength calibration. The DAD may have the initial parameters changed during a run by a time-based program. Users can program wavelength changes, the threshold for peak-controlled spectral acquisition, the spectral data acquisition rate and the spectral acquisition mode changes in the time table.

Initial parameters that may be set for the DAD include signal wavelengths and reference wavelengths, spectral acquisition mode, signal sampling rate and autobalance.

A spectral monitor window is included in the ChemStation to show the spectra being acquired both during and between analyses. Users may interactively change the absorbance and wavelength of this display.

The Agilent ChemStation for CE may be configured for direct connection of the capillary outlet to a mass spectrometer.

Specialized CE Data Evaluation

Each configured CE instrument has the following data evaluation features beyond the capabilities of the basic chromatography ChemStation:
• technique specific terminology,
• capillary specifications,
• full spectral evaluation for one instrument as described in the 'LC diode-array spectral evaluation' section on page 30,
• technique specific reports including a mobility report that compensates for the velocity of the compounds migrating through the detector cell, and
• calibration based on velocity corrected peak areas.
• calibration based on:
  → *Protein Molecular Weight* using the migration times of standard with known molecular weights to calculate sample molecular weights.
  → *DNA Base Pair* using the migration times of standards with a known number of base pairs to calculate sample base pairs.
  → *cIEF pl* using the isoelectric point (pl) of a known protein standard to calculate the pl of an unknown.

Acquisition with the Agilent ChemStation for A/D (G2072AA) and the Additional A/D Instrument Module (G2073AA)

The A/D ChemStation and additional A/D interface acquisition module controls and acquires data from Agilent 35900E dual channel interface. These interfaces allow the ChemStation to acquire data from instruments that are not capable of being interfaced for data acquisition through the GP-IB system or LAN. One or two analog signals per instrument may be configured; if only one is used the other is available for use with another instrument running on another timebase. Data may be acquired at up to 100 Hz per signal.

The user may also define the units for acquisition and their relationship to the voltage signal (units/volt). The Agilent 35900E interfaces offer external event control through digital TTL (transistor-transistor logic) signals, each of which are given specific state (high and low) names, that may be time-programmed before, during and after an analysis. The Agilent 35900E can be configured for up to eight TTL signals for each independent channel.

Additional Data Evaluation Modules

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

- LC diode array detector (DAD) spectral evaluation module (G2180AA)
- Agilent ChemStore sample organization and results database module (G2181BA)

LC Diode Array Detector (DAD) Spectral Evaluation Module (G2180AA)

UV-visible spectra, acquired using a diode-array detector, may be graphically selected from a chromatogram signal for visual inspection and comparison or may be used for peak purity determinations, wavelength optimization and component identification through spectral libraries. The spectral library functionality can be extended to automatic identification of components in up to four user-defined spectral libraries based on peak or target compound identification.

Interactive Spectral Processing

Users may graphically select spectra from a chromatographic signal for visual inspection and printing. The spectra are displayed in a separate spectral window and may be overlaid for comparisons. The user can select spectra in the following modes:
- individual spectrum,
- peak apex spectrum,
- average spectrum over a graphically defined retention time range,
- range of spectra, and
- all spectra over a peak

The user may also select how the spectra are processed when they are extracted. The available options include:
- setting the subtracted reference spectrum or spectra,
- limiting the extracted wavelength range,
- customizing the spectral and reference display, and
- setting the spectral processing. Options include setting a smoothing and splining factor, logarithmic processing and derivative order.

Peak Purity Determinations

Peak purity may be determined interactively on a peak by peak basis, for all the peaks from a certain data file, or automatically at the end of each analysis as part of the method. Users may optimize peak purity processing for accuracy or performance by setting preferences relating to:
- the number of spectra used over a peak,
- the wavelength range used for the purity determination,
- the reference spectra,
- the purity threshold,
- spectral processing including logarithmic, smoothing and splining factors and derivative order.

The purity components are calculated and displayed. These include the spectra, the spectral differences, the signals, a signals-based ratiogram, similarity and threshold curves.
Similarity curves give the most detailed information about a peak's purity. User selected or average spectra are compared with all the other spectra acquired during the peak's elution and the resulting spectral comparison factors are plotted as the similarity curve. For a perfectly pure peak the similarity curve will be a straight line corresponding to a theoretically pure compound. Impurities will cause a deviation from this ideal line. The similarity curves are plotted with reference to the theoretically pure line and the user-defined purity threshold. The similarity curve gives the best indication of any impurities that occurred in the peak as it eluted.

The deviation of the similarity curve from the ideal theoretically pure value is influenced by both compound impurities and spectral noise. The user-defined purity threshold may be replaced by a system calculated threshold curve based on the signal-to-noise ratio of the peak in question. The noise sample may be user selected and, if truly representative of the spectral noise when the peak eluted, compensates for any deviation of the similarity curve, from the theoretically pure value, attributable to spectral noise.

Wavelength Optimization using the Iso-absorbance Plot

UV-visible spectra, acquired continuously during an analysis, can be used to determine the optimum signal wavelengths and bandwidths for routine detection by a signal-based method. The iso-absorbance plot displays the acquired spectra as a color-contoured map of wavelength against retention time together with areas for the display of both signals and spectra defined by the position of the cross-hairs on the iso-absorbance plot.

The iso-absorbance map can be used in four modes:

- Quick view mode allows users to view and compare spectra and signals by moving a crosshair over the area of the contour map. Spectra and signals are continuously extracted and updated in the display areas. The extracted spectra and signals may be frozen in the display areas for comparison purposes.
- Zoom mode allows users to zoom into areas of interest on the iso-absorbance map.
- Signals mode allows users to extract a particular signal, with a graphically determined bandwidth, into the chromatographic window for routine data processing such as integration and quantification.
- Spectral mode allows users to extract spectra into the spectral window for further processing.

The iso-absorbance plot is typically used during method development to explore the sample's response at different UV-visible wavelengths in order to determine the optimal detection wavelengths and bandwidths through experimentation with the integration and quantification processes.

Users can define the contour color schemes and retention time and wavelength ranges for display.

Three Dimensional Plot

UV-visible spectra acquired continuously during an analysis, can be displayed as a three-dimensional plot. The display may be graphically adjusted by the users in the time, intensity and wavelength domains. The resolution of the plot is selectable.

- The orientation of the plot can be adjusted graphically. The orientation of the display is not restricted in any dimension.
- The plot may be printed.
- The color scheme used in the plot may be selected from a number of choices.

Spectral Libraries

Spectral libraries allow users to positively identify compounds by comparing the spectra of peaks in the sample to libraries of spectra derived from analytical standards.

The ChemStation allows users to use libraries both interactively and automatically. The ChemStation can manage an unlimited number of spectral libraries each with up to as many entries as there is available system memory (typically hundreds of entries). Libraries may be loaded and searched by selecting individual spectra from a chromatogram and searching the library for the best matches. The library search may be constrained by specifying a search template that allows the user to define a retention time window and include the informational data associated with each library entry. For example, the applicable retention time can be constrained to ± 5 % of the library retention time and the entry names must start with the letter 'B'. The search results may be displayed on the screen and printed.

Users may build their own libraries by analyzing known substances under defined analytical conditions, creating a new library and entering the individual spectra and the information fields to describe the entry into the library. Library entries may be added, deleted, edited, viewed or printed. Details of each spectrum in a library including absorbance and wavelength data may be examined.

Automated Spectral Library Search Reports

Automated spectral library search reports allow users to automatically identify and quantify unknown samples based on the positive identification from up to four separate spectral libraries. Search criteria may be specified for each library separately through a library search template that allows users to constrain the search both in the retention time and library entry identification parameters.

One of three search modes may be selected :
- Standard search mode identifies each integrated peak in the chromatogram from the library.
- Target analysis from the calibration table limits the library search to those library entries identically named in the calibration table. Identification may be further constrained through the use of the library template to restrict other search criteria such as the retention time window. After positive identification, quantification proceeds according to the data in the calibration table
- Target analysis from the library uses the library entries to identify peaks in the chromatogram that are within the RT window specified for the particular library entry. This mode differs from the standard search mode in that it excludes peaks whose retention times are not covered by library entry time windows.

Consequently it is typically faster than the Standard search, especially if there are many more peaks in the sample than there are entries in the library. After positive identification, quantification proceeds according to the data in the calibration table.

The calculation of the peak purity factor may be included as part of the library search.

Report styles can be selected to produce simple library search reports or a combination of library search and standard performance reports described above.

Spectral Data Import and Export

The ChemStation spectral module can import spectra stored in Agilent's .WAV format files, from the HP 8452 and Agilent 8453 spectrophotometers, and industry standard JCAMP spectrum files.

The ChemStation is data file compatible with Agilent's ChemStation for UV-visible spectroscopy running in the Windows environment. Both DAD and UV-visible spectra may be exchanged between the two systems either as ChemStation register files or through the Windows clipboard.

LC/MSD Deconvolution and Bioanalysis Module

The LC/MSD deconvolution and bioanalysis data evaluation module adds capability for deconvolution of mass spectra containing multiply charged ion series as produced by API-Electrospray ionization, and for evaluation of MS data from biopolymers such as proteins, peptides, and oligonucleotides.

Deconvolution Software

When biopolymers such as peptides, intact proteins, and oligonucleotides are analyzed with the Agilent 1100 Series LC/MSD using API-Electrospray ionization, the resultant mass spectra often consist of a series of multiply charged ions. The deconvolution software can be used for determination of the molecular weight of analytes from such multiply charged ion series. The deconvolution capability can be used interactively, or deconvolution can be performed automatically at the end of each analysis as part of a method.

When used in interactive mode, a specialized toolset allows the user to graphically select mass spectra from a mass chromatogram for deconvolution, to adjust the parameters for deconvolution, and to perform deconvolution. The software displays the original chromatogram, raw mass spectrum, charge-series separated intermediate spectrum, and final molecular weight results. When multiple

components are present in the results, the user can choose which component(s) to display, and can delete one or more components and perform additional mass peak detection and deconvolution steps on the residual data. A deconvolution results report can be viewed and/or printed.

The user can also mark a mass spectrum with the expected location of mass peaks corresponding to the multiply charged ion series for a user-specified molecular weight and charge state range.

Bioanalysis Software

The bioanalysis software provides specialized data evaluation for mass spectral data acquired from analysis of biopolymers such as proteins, peptides, and oligonuclotides. Using the graphical interface, users can process data interactively. Bioanalysis data evaluation can also be performed automatically at the end of each analysis as part of the method.

Bioanalysis software capabilities include:

- user-definable building block (e.g. amino acid, nucleotide) sets,
- copy sequence data in text form from the Windows clipboard into the sequence editor,
- copy and paste sequences or subsequences between two instances of the software,
- molecular weight calculations in multiple modes (average, most abundant).

- prediction of m/z values for intact and cleaved biopolymers (e.g. intact proteins and peptide fragments),
- support of intra-chain and inter-chain links (e.g. disulfide bridges in proteins),
- individual amino acids can be flagged as being chemically modified,
- automatic biopolymer digestion to simulate enzymatic cleavage,
- manually selectable digest cleavage,
- user-customizable building block modifications and digest regents,
- calculation of collision induced dissociation (CID) masses for biopolymers (e.g. peptides) ,
- automated or manual matching of mass spectral data from a biopolymer analysis with predicted m/z values for intact and /or cleaved biopolymer fragments (e.g. peptide map fragments),
- automated or manual mass matching report generation, and
- isotopic profiling for a specified m/z value or molecular formula at a user-selectable mass resolution value.

LC/MSD Easy Access Software Module

The Molecular Weight Confirmation System for Medicinal and Synthetic Chemistry is based on the LC/MSD Anyone Software and provides a quick, easy, and complete solution to the problem of confirming molecular weights, whether for a single sample or for a batch of a few samples. This system makes confirmation of syntheses fast and easy, even for users without mass spectrometry expertise. It answers the key question "Did I make the right compound?'

In a "walk-up" mode, users simply input pertinent sample information, place samples in the external tray and the system does the analysis, including printing a report confirming the MW of the expected compounds.

LC/MSD Molecular Weight Confirmation Software Module

The Molecular Weight Confirmation System for Combinatorial Libraries is a complete LC/MS solution for the rapid molecular weight confirmation of combinatorial libraries and is based on the CC Mode Software module operating within the ChemStation Software. With this system the user can rapidly identify the products of combinatorial syntheses and estimate their purities before screening. As a result, there is fast validation of combinatorial methods and efficient

confirmation of large combinatorial synthesis projects. Drug leads can then be generated and new leads optimized without the need for extensive mass spectrometry expertise

Agilent ChemStore C/S Database Client Software

The Agilent ChemStore C/S databse client software G2181BA may be added to any Agilent ChemStation configuration. The specifications for this product may be found in the dedicated "Agilent ChemStation Plus Specifications" document.

Agilent Chemstation Plus Security Pack

The ChemStation Plus Security Pack is a module of the Agilent Plus Series designed to support the requirements of 21 CFR Part 11. In the Agilent ChemStation the ChemStation Plus Security Pack modifies data analysis and provides advanced data management with regard to supporting the requirements for electronic records and electronic signature. The specifications for this product can be found in the "Agilent ChemStation Plus Specifications" document.

Agilent ChemStation Plus Method Validation Pack

The Method Validation Pack is an add-on software module for ChemStation Plus and provides a fully validated, off-the-shelf software solution that supports labs in performing the entire method validation process from planning through to presentation of the final report – without the problems associated with data transfer. The specifications for this product can be found in the "Agilent ChemStation Plus Specifications" document.

Networking

The software has been successfully tested for compatibility with the standard networking components of the Windows environment. The software will run at the same time as other network software and computer applications written for, and adhering to, the recommended programming practices of the Microsoft Windows operating environments.

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.

The Agilent 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 Agilent ChemStation installation in one work environment.

Documentation

The documentation set has specific components designed for:

- installing and learning the Agilent ChemStation software,
- using the Agilent ChemStation software,
- understanding the principles of how the software works, and
- customizing the Agilent ChemStation.

Installing and Learning

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

Each Agilent 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 on-line information are designed for the routine user.

The ChemStation includes comprehensive, Windows-style, context sensitive and indexed on-line 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 in the users own documentation, or printed.

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 directly linked to the detailed on-line help information.

Understanding the Principals

The *Understanding Your ChemStation* manual documents the principals 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 using the command set.

The primary reference manual, *A Guide to Macro Programming* (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 on-line, the users can copy the examples and command syntax directly into their own macro source files.

Agilent Technologies