System Aids Analysis of Compound Collections

A fully automated high-throughput HPLC-MS system aims to shorten the drug discovery cycle with minimal human intervention.

Simon Chatwin; David T. Chow; Edward Maliski, PhD; Wally Talen, Gregory Woo; Yining Zhao, PhD; and David J. Semin, PhD

Chatwin, Chow, Maliski, Woo, Zhao, and Semin are members of research departments at Amgen Inc., Thousand Oaks, Calif. Talen is a former Amgen employee.

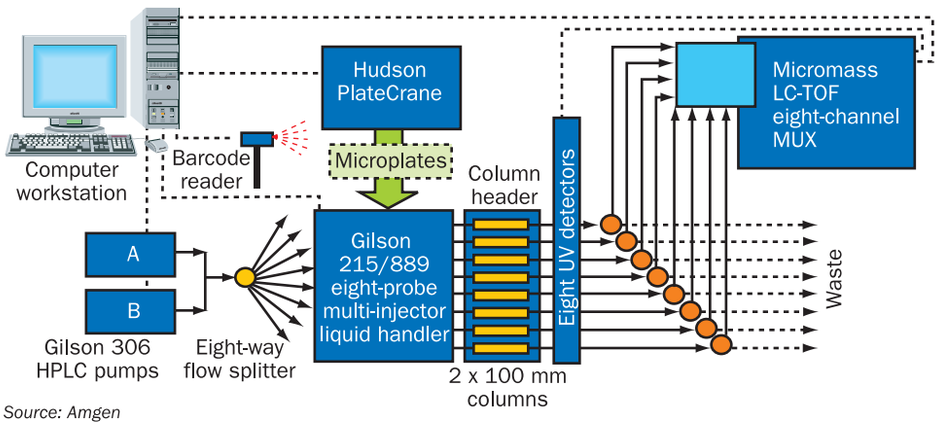
There is an increased need for high-throughput structure confirmation and purity assessment with today's extensive use of combinatorial chemistry to create large libraries of compounds. High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) techniques continue to be the preferred method of choice because of the speed, versatility, reliability, and sensitivity for detecting a wide range of pharmaceutical-like compounds. Current trends within the pharmaceutical industry are toward reducing run times by use of rapid solvent gradients, column-switching techniques, and use of parallel analyses by parallel setups.

In 1999, Micromass Inc., Manchester, UK, launched a parallel MS system on an orthogonal time-of-flight (TOF) platform, which is comprised of a multi-indexed rotating sprayer (MUX technology) as the electrospray source. The indexing of sample streams on the TOF instrument affords high sampling rates for up to eight channels. Gilson Inc., Middleton, Wis., Micromass Inc., and Hudson Control Group, Springfield, N.J., collaborated to develop a fully robotic high-throughput HPLC-MS system. The purpose of the system is to provide unattended, continuous operation of an automated parallel HPLC-MS system. The system is comprised of a microplate robotic handler (Hudson), an eight-channel liquid autosampler (Gilson), an eight-channel UV-Vis detection system (Shimadzu Inc.), and an eight-channel MUX electrospray source on an LCT-TOF mass spectrometer, all controlled by a central computer workstation.

Communication among the different components is coordinated by a process flow diagram that includes all aspects of the system. Communication protocols with the Gilson 306 HPLC pumps and eight-channel liquid handler are achieved via standard Gilson serial I/O channel communication to a 506C interface box. The 506C interface box is connected to the central computer workstation via RS-232 communication.

The communication between the barcode reader from Symbol Miniscan, Bohemia, N.Y., and the Hudson PlateCrane to the computer workstation is via RS-232 communication. Analog outputs (0 to 1 V) from the eight UV-Vis Shimadzu detectors are connected to the analog input channels on the back panel of the Micromass LCT and the auxiliary MUX box used to control the MUX sprayers. The output from the Micromass LCT-TOF is coupled by a fiber-optic cable to a high-speed sys-

Components Integrated With Workstation



Source: Amgen

This system overview shows the integration of components to the single computer workstation. It requires little human intervention, and offers high sampling rates.

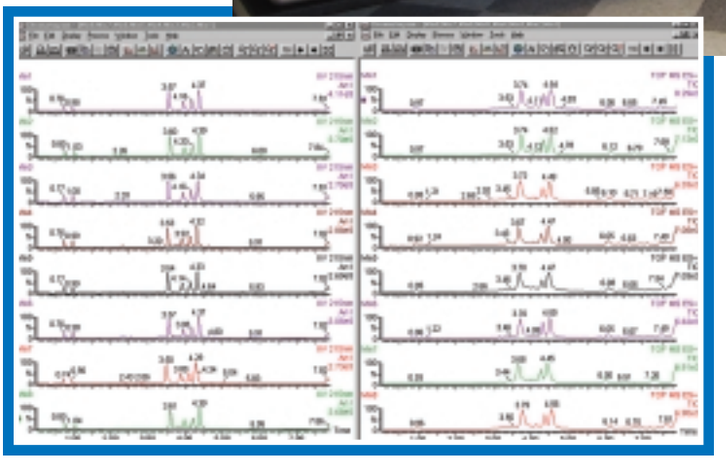
tem controller that is connected to the computer workstation via an Ethernet connection.

THE FLOW OF INFORMATION

It is essential to understand the information data-flow process to obtain a reliable and robust high-throughput system with minimal user intervention. The PlateCrane acts as the initiator of data acquisition. The first step in this process involves the PlateCrane picking up a microplate from one of its stacks and moving the plate to a position in which the barcode can be read.

For Amgen's particular configuration, there are six stacks. Each stack can accommodate 30 microplates or nine deep well plates. The barcode is read via a barcode reader through a serial RS-232 interface to the central computer workstation. Within Hudson's Total Control for Windows (TCW) operating environment, a program called get_data.tcl is used to read data from the Oracle database. Hudson's TCW package is flexible in the logical programming and syntax structure and is easy for the end user to modify.

The Multiplexed high-throughput HPLC system occupies minimal lab bench space. From left is the Gilson 215 liquid handler, Hudson PlateCrane, Shimadzu UV-Vis detectors, and Micromass LCT-TOF. The Gilson 306 pumps are below the Gilson 215.



Multiple representative chromatograms at 215 nm for a standard four-component mixture. The panel on the right shows the corresponding total ion chromatograms.

Once access to the Oracle database is established, the information from the barcode string is used to access the platemap from the Oracle tables. The platemap contains information, such as a unique Amgen sample identifier, well position, formula weight, and molecular weight, for all compounds contained on that plate. The TCW package creates a local copy of the platemap without compromising the original integrity of the database.

A second program within TCW, called mmass.tcl, is used to generate an importable text file platemap (*.olb) that the MassLynx software can interpret. Once the file has been created from the Hudson program, it is automatically copied to the AutoLynx subfolder within the Micromass software domain. MassLynx software can be set up to continuously poll or monitor the AutoLynx folder to detect if any new files exist.

If AutoLynx detects the presence of a new file, it will automatically attempt to import it and create a sequence list in MassLynx. For Amgen's setup, there are two folders within AutoLynx called "processed" and "failed." OpenLynx.rpt files will be written to these folders, depending on whether there were any errors. If an error occurs, the mmass.tcl TCW program records error information in a log file based on the current date.

The next step is for the PlateCrane to move the microplate onto the deck of the eight-channel liquid handler. It takes approximately 30 sec to pick up a microplate, read the barcode, and generate a *.olb file. At this point, the software takes control via AutoLynx and activates the HPLC Gilson 306 pumps and 215 liquid handler to start the injection cycle.

In the injection cycle, Gilson parameters and Micromass LCT parameters are stored in LCMethod, MSMethod, and MSTune files, respectively. The Hudson software can be used to prompt the end user for which file they would

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like to use for a particular acquisition. At this point in the cycle, the Micromass software is in control and data acquisition starts.

SELECTING FUNCTIONS

There are five main functions that are in the main menu. Function 1, or run method, is used to execute the start of an acquisition with a window prompting the user to specify the number of plates in each PlateCrane stack, followed by an option to start or terminate the acquisition. Function 2, or edit method, is used to specify the sequence of movements in a method. Function 3, edit position list, is used to set limits and coordinates of PlateCrane components. Function 4, calibrate PlateCrane, is used to teach the positions and movements for methods. Function 5, or change PlateFormat, is used to specify either 96- or 384-well plate format.

The LC portion of this device consists of the Gilson 215/889 liquid handler, Gilson 306 pumps, eight HPLC columns, eight Shimadzu dual-wavelength UV-Vis detectors, and an eight-channel MUX box on the front of the Micromass LCT-TOF mass spectrometer. For these experiments, the 306 pumps were set up to operate at 3.2 mL/min and were split with an eight-way flow splitter prior to going into the injectors on the liquid handler. All eight valves on the Gilson 889 are cycled simultaneously with the output flow going to eight 2.0- × 150-mm YMC-AM columns housed in an Alltech 530 column heater. The effluent from the columns goes into the eight Shimadzu UV-Vis detectors and the flow is split again in order to provide 50 µL/min/channel flow into the MUX electrospray source.

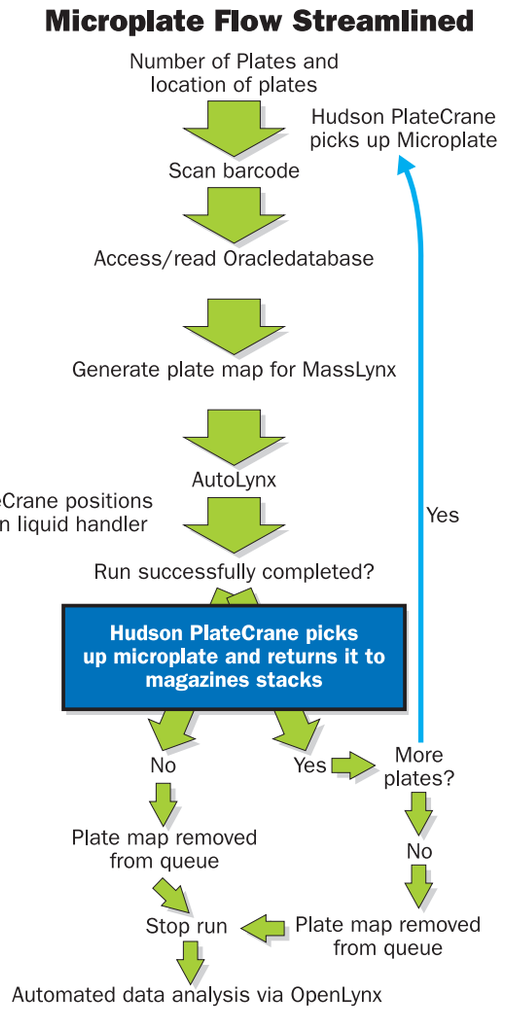
Test solutions are injected into the LCT-MUX system before each set of samples is analyzed. As a guide, the relative standard deviations of retention time and relative peak area or height among all eight channels should be less than 5%, except for total ion chromatogram (TIC) signals, since the final purity assessment is only based on the UV trace. Results outside these limits may indicate a problem with the system and corrective measures should be taken before proceeding with the analysis.

MANAGING THE DATA

The most important aspect on setting up a fully automated HPLC-MS system is to address the file management and subsequent data processing. Amgen utilizes the Micromass OpenLynx software package to adjust integration parameters and create the text format *.rpt files during data acquisition. Once a batch has been acquired and processed, a Visual Basic application moves and sorts the raw Micromass folders (*.raw), generating one folder per plate of generated text files (*.rpt). Thereafter, another Visual Basic application converts and extracts the rpt files into an Excel user-friendly format.

Columns stored in the Excel format include UV intensity and maximum UV intensity; TIC purity, intensity, and area; base peak maximum; and other ions of significance above a predetermined threshold. By storing all this information it allows the analyst to determine the system integrity and sensitivity across all eight channels. Long-term archival and retrieval of the results are managed within an Oracle-based analytical information management system. A Web-based analytical information management system allows end users to query and dynamically browse individual HPLC-MS traces with compound structures and physical properties displayed on the page.

This system is very amenable to unattended high-throughput HPLC-MS analysis. We have addressed and validated all aspects, from microplate handling through data processing. The system has proven to be reliable and flexible enough to meet many demands of quality control within drug discovery. We have routinely used this system to characterize combinatorial libraries, validate "actives" from high-throughput screening, and for general quality control screening of our corporate compound collection. With this capacity described, we are able to fully process 1,000 to 2,000 HPLC-MS analyses/day, thus shortening the drug discovery cycle.



The flow process shows the streamline approach from the reading of barcodes to the generation of data. The software assists in automation.

For more information, contact:
Amgen Inc.
805-447-1000
www.amgen.com