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Shimadzu's newsletter for HPLC users

LC WORLDTALK

Volume 4, Number 1, March 1999

This issue of "LC WorldTalk" features several articles selected especially to enlighten Shimadzu HPLC users worldwide about recent trends, solutions, and products of particular interest to the global HPLC community.

The **Analysis** and **Real World Solutions** articles focus on advances in automated procedures to increase the efficiency of HPLC systems.

In the area of instrument control and data handling, Shimadzu is proud to introduce CLASS-VP ™ 5.0, the newest addition to the VP series HPLC system. A tour of the powerful control, manipulation, validation, and presentation features incorporated in this package is provided in the **Products** section on Page 6.

Finally, learn how Shimadzu is meeting the challenge of the **Year 2000** date change on page 12.

Contents of this issue

"LCWorldtalk" is a complimentary newsletter from Shimadzu designed to keep you apprised of the latest HPLC techniques and applications.

Available to all HPLC users worldwide, "LCWorldtalk" grew from the popular "LCtalk," the newsletter introduced in 1984 and now distributed to more than 30,000 users.

For over 120 years, Shimadzu has been providing solutions for science. We are proud to offer "LCWorldtalk" as an additional vehicle for communicating with all HPLC users in the global community.

Automated Analysis of P450 Enzyme Activity in Human Liver Microsomes with the Shimadzu LC-10Ai Bioinert HPLC System

by Steven M. Wishnies, Sanjeev Thohan, Peter Ratsep, and Dario Fiore

Introduction

Over the past decade, the large number of compounds synthesized by combinatorial chemistry increased the demand to automate the identification, purification, and throughput of potential drug candidates. Now, automated compound synthesis procedures and improved chemistry challenge other areas of the drug development process. New bottlenecks are emerging in the pharmaceutical industry, for example, in the biological screening stage of drug candidates.

The screening stage is one of the most important steps in drug development. Ideally, compounds are designed to eliminate a disease or its symptoms, while producing the least amount of systemic toxicity. The potentially beneficial and toxic effects of drug candidates are usually characterized in-vitro prior to clinical trials. In-vitro metabolism and toxicity studies are designed to closely mimic human in-vivo biotransformation. For in-vitro metabolic screening, enzyme-enriched tissue fractions, such as microsomes, are commonly used. The major drug metabolizing enzymes responsible for the bioactivation, detoxification, and initial elimination of chemical compounds are the Cytochromes P450; these can be isolated and screened using the microsomal fractions of human and animal tissues.

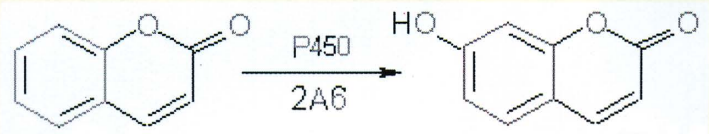

Figure 1 Human liver microsomal metabolism of coumarin (left) and 7-hydroxycoumarin metabolite (right) by Cytochrome P450, CYP2A6.

The Cytochromes P450 are a supra family of proteins that catalyze the oxidative metabolism of xenobiotics, which are biologically and synthetically produced chemical compounds found in pharmaceuticals, foods, plants, pesticides, environmental toxins, and human and animal endogenously produced substrates (hormones, steroids, etc). Isoforms of this multigene family of enzymes are tissue- and species-specific and determine the biotransformation of therapeutics in humans and animals. Although these enzymes are ubiquitously distributed throughout different tissues and organ systems, they are primarily located in the liver and can be further concentrated and isolated in microsomal preparations. The use of discarded non-transplantable human liver tissue from organ donors is an invaluable source of microsomes for the in-vitro metabolic screening and profiling of compounds in the drug discovery pipeline.

Microsomal P450 activity (known as Phase I oxidative metabolism) can be phenotyped or probed using different xenobiotics that are specific for particular isoenzymes. For example, coumarin, a probe for coumarin 7-hydroxylase activity, has been unequivocally proven to target a specific P450 isoenzyme (2CYP2A6) in human liver[1] (Fig.1).

Determining P450 catalytic activity in-vitro is an important part of drug metabolic screening. It requires the repetitive steps of liquid dispensing, sample pretreatment and timed reactions for subsequent analysis by HPLC. However, these types of analyses necessitate the use of physiological buffers, co-factors and proteins which can damage the analytical system; conversely, the system might also affect the sample. Therefore, the analytical HPLC system should be corrosion-resistant, as well as automated, for high sample throughput.

This article describes the development and methodology of a new assay for the automated analysis of a well-characterized P450 isozyme, coumarin 7-hydroxylase activity, in human and rat liver microsomes. The analysis was conducted using the Shimadzu biocompatible HPLC system, and the dual, deep-well microtiter plate (MTP) option.

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Experiment

Instrument

The bioinert HPLC system configuration consisted of the Shimadzu SCL-10A system controller, SIL-10Ai auto injector (equipped with reagent vial rack and the dual deep-well MTP optional rack for microtiter plates), SPD-10AV UV-VIS detector, LC-10Ai pump, CTO-10A column oven, and a GT-104 mobile phase membrane degasser. Initially, an FCV-10AL low pressure mixing valve was used to optimize the proportion of water to methanol in the mobile phase. Data was collected with a data station running the CLASS-VP™ 4.2 chromatography software (Fig. 2).

Reagents

The methanol used for mobile phase was HPLC grade, purchased from Burdick and Jackson (Muskegon, MI, USA). All other chemicals used for preparing and incubating both rat and human liver microsomes were the purest available (analytical grade), purchased from Sigma Chemical Company (St. Louis, MO, USA). Pooled Human liver microsomes were generously donated by the Analytical Gift Foundation (Laurel, MD) and were prepared from 5 different non-transplantable human livers. Ages of the donors ranged from 20 to 40 years; the cause of death was usually closed-head injury resulting from motor vehicle accidents. Three livers were from male organ donors and two from females. Rat liver microsomes were prepared from male Sprague Dawley rats (Charles River Laboratories) weighing between 180-220 g. Rat Liver microsomes were kindly donated by Dr. Thohan at the University of Maryland at Baltimore, Department of Pharmaceutical Sciences (Baltimore, MD).

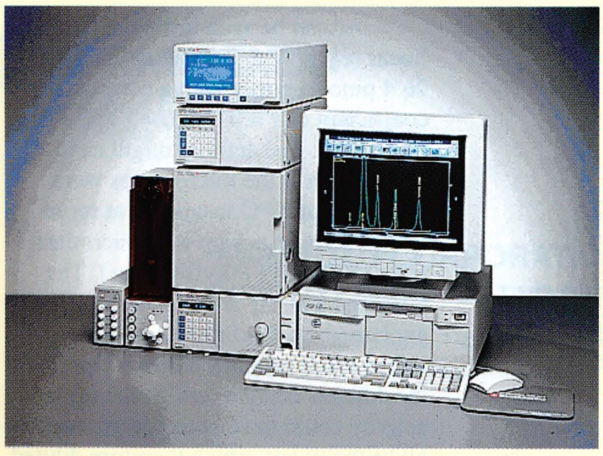

Figure 2 The LC-10Ai HPLC configuration linked to a data station with CLASS-VP™ software.

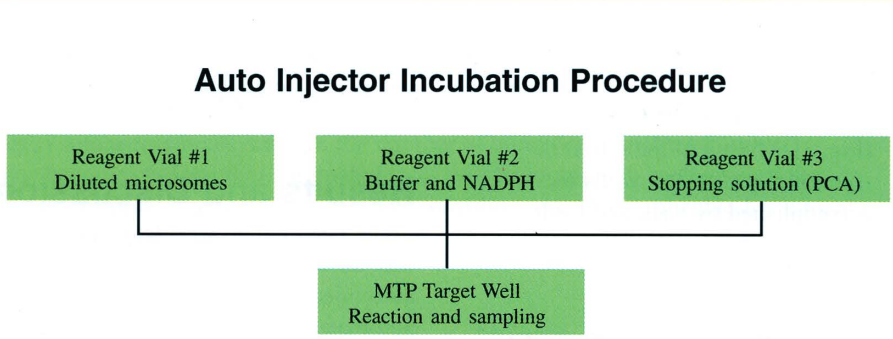

Figure 3 Microsomal incubation pretreatment procedure for the SIL-10Ai bioinert auto injector.

Microsomal Preparation

Liver microsomal fractions were prepared according to the methods of Van derHoeven and Coon[2] and Halpert[3]. Briefly, liver tissue was weighed and added to four volumes of buffer consisting of 50 mM Tris, 0.1 M KCl, 1.0 mM EDTA and 20 µM BHT (pH 7.4). Liver samples were then subjected to three 10-second bursts from a Polytron homogenizer. The homogenate was then centrifuged at 10,000 x g for 20 minutes at 4°C. The supernatant was then centrifuged at 100,000 x g for 60 minutes at 4°C. The supernatant was then discarded and the resulting pellet (microsomal pellet) was resuspened in a storage buffer (1.0 mM Tris, 10 mM EDTA, 20% glycerol v/v, and 100 µM phenylmethylsulfonyl fluoride), aliquoted and frozen at –80°C until use. Prior to freezing, the microsomal protein content was assayed according to the dye binding method of Bradford[4].

Microsomal Incubation Strategy

The objective of this experiment was to automate the microsomal incubation procedure or kinetics of the P450 assay and the subsequent HPLC analysis of the metabolite produced by the microsomes. Essentially, human or rat microsomes (1mg/mL protein) were incubated with a 100 µM coumarin substrate in a reaction volume of 1mL (50 mM Tris buffer, pH 7.4) for a total of 30 minutes; the reactions were automatically stopped every 5 minutes with the addition of concentrated perchloric acid. The auto injector pretreatment file specified the addition of the appropriate volume of coumarin substrate/500 mM NADPH /50 mM Tris buffer solution to the target well of the microtiter plate, followed by the addition of human microsomes to start the reaction. After the appropriate incubation period, the

auto injector dispensed PCA from a third reagent vial to the target well to stop the reaction. Afterwards, the upper portion of the supernatant was analyzed for the 7-hydroxycoumarin metabolite (Fig. 3). Mixing of the different solutions in the target wells was accomplished by back and forth aspiration of the well contents with the auto injector needle.

HPLC Separation Conditions

A simple isocratic method was developed to separate coumarin from its major metabolite, 7- hydroxycoumarin. Metabolites are generally more polar than their parent compounds; therefore, using a current reversed-phase method, the metabolite should elute earlier than the parent compound. Precipitated protein settled prior to injection (volume: 10 µl), so the sample needle was programmed to sample from the top of the supernatant. The assay was developed using the new PEEK ™ Betasil Basic column (150 x 2.1 mm) (Keystone Scientific, Inc.). The separation was optimal at a 50/50 methanol/water mixture with a flow rate of 150 µL/minute, with a constant column temperature of 40°C. Detection of both 7-hydroxycoumarin and coumarin was optimal at a wavelength of 320 nm. The compounds eluted at 4.3 and 5.3 minutes, respectively (Fig. 4).

Results and Discussion

The method was developed to take advantage of Shimadzu's advanced auto injector pretreatment files to automate both the microsomal incubation and subsequent HPLC analysis of P450 activity in human and rat liver microsomes.

Naturally, the microsomal incubation times will vary from substrate to substrate, requiring different pretreatment programs. In addition, one must carefully consider the analytical run time when planning the pretreatment file for automated kinetics. The shorter the kinetic incubation time, the more complicated the pretreatment file becomes; therefore, the incubation strategy should be revised for different substrates. In this case, the coumarin incubation and analysis was possible with 5 minute kinetic time points.

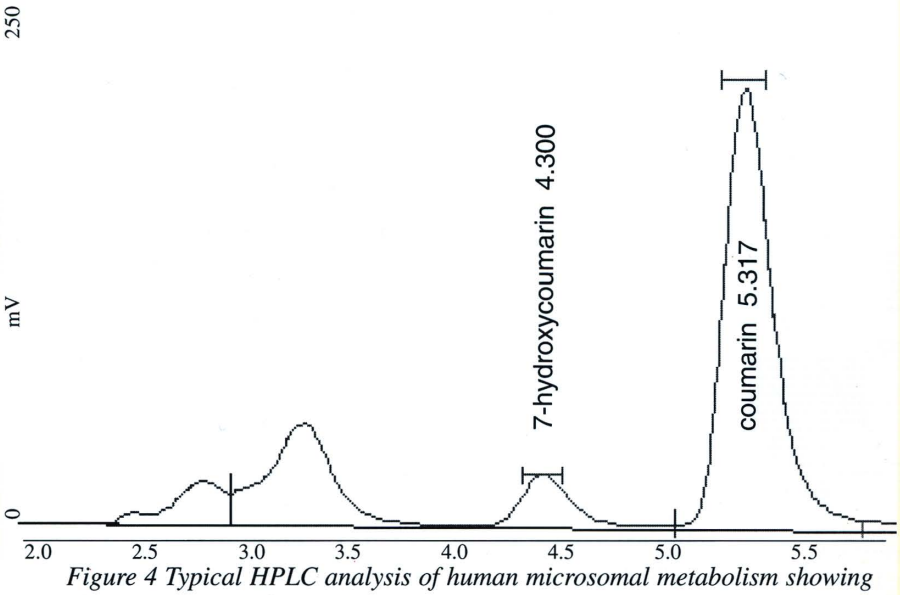

Figure 4 Typical HPLC analysis of human microsomal metabolism showing clear separation of coumarin and 7-hydroxycoumarin metabolite. Quantitation of 7-hydroxycoumatin was accomplished using an authentic standard.

Rat microsomal incubations failed to yield any appreciable amount of 7-hydroxycoumarin. This indicates that the P450 enzyme is not present in this particular species of rat. The baseline was flat where 7-hydroxycoumarin elutes, and the full compliment of the parent substrate could be accounted for (data not shown) even after 30 minutes of incubation. It is also possible that the microsomal activity was not present due to problems with microsomal preparation or storage. Other more global microsomal assays were performed to check this. Ethoxycoumarin o-deethylation was checked and found to have an enzymatic activity or rate of metabolism of 238.8 +/- 62 pmol/mg/min. Cytochrome C reductase was also determined and was 67.7 +/- 7.4 nmol/mg/min (activity of mg of microsomal protein present). The level of total P450 appeared to be normal. Therefore the rat microsomal preparation was active, but had no P450, CYP2A6 activity. The human microsomal preparation was also active, and exhibited coumarin hydroxylase activity even after 5 minutes of incubation.

The human microsomal protein coumarin hydroxylase activity was linear during the 30 minute course of incubation. Three injections were performed per time point.

As can be seen in Figure 5, the linear reaction progressed at a rate of 76.4 pmol/mg/min with a correlation coefficient of r = 0.998 (n=3 injections/timepoint). The activity might have been higher if the microsomes were incubated at 37°C instead of at room temperature in a deep well microtiter plate (Whatman Polyfiltronics). The low final activity level could be explained by the differences in the individual levels of CYP2A6 present in the different donor livers. Some of the activity could have been low in some or all of the livers, which therefore diluted the final activity when the microsomes were pooled. However, the main objective was to qualitatively demonstrate the feasibility of automating microsomal incubations.

Conclusion

It has been demonstrated that the microsomal incubation procedure (kinetics) and subsequent HPLC analysis can be automated. The Shimadzu LC-10Ai bioinert HPLC system offers the added advantage of efficiently operating under more stringent running conditions (exposure to concentrated salt buffers, protein and acids).

With the advent of combinatorial chemistry and automated synthesis, the number of compounds in drug discovery/development has increased exponentially. It has become obvious that automated tools will be required at each level of the drug-to-market process. This method serves as an example of automating metabolic profiling, one tool applicable for screening drug candidates at the developmental level of drug discovery.

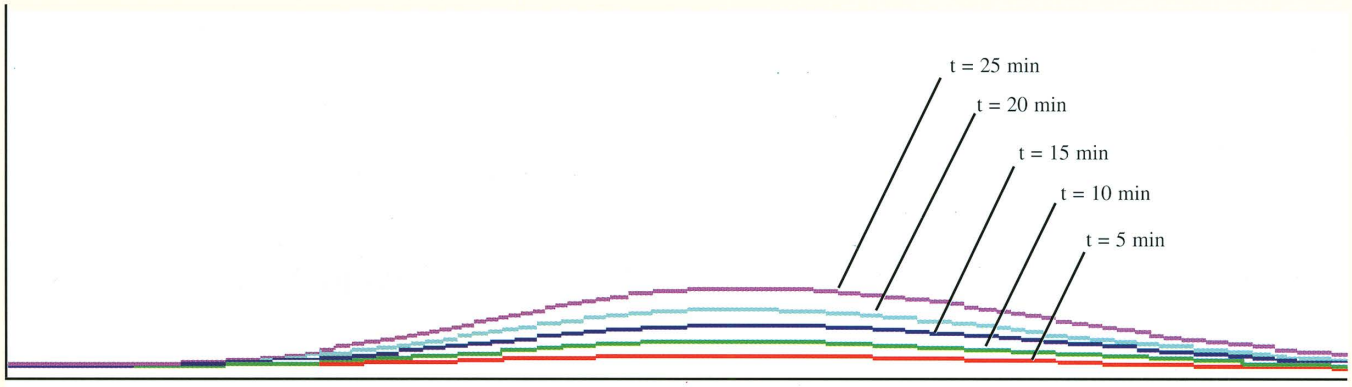

Figure 5 Overlaid chromatograms of the human microsomal metabolite peak production (7-hydroxycoumarin) after 5, 10, 15, 20 and 25 incubation periods.

References

[1] Draper, A. et al., 1997. Inhibition of coumarin 7-hydroxylase activity in human liver microsomes. *Archives of Biochemistry and Biophysics*, V.341 No.1, May 1. pp. 47-61.

[2] Van der Hoeven, T.A. et al., 1974. Preparation and properties of partially purified cytochrome P-450 and reduced NADPH P-450 reductase from rabbit liver microsomes. *Journal of Biological Chemistry*. 249:6102-6112.

[3] Halpert, J. et al., 1983. Suicide inactivation of rat liver cytochrome P-450 by chloramphenicol *in vivo* and *in vitro*. *Molecular Pharmacology* 21:445-452.

[4] Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analytical Chemistry*. 72:248-254.

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Each regional office has its own website, which can be accessed from the global page by clicking on the appropriate area. Be sure to visit your area's unique site for regional information.

PRODUCTS

Validation & Productivity

CLASS-VP™ v. 5.0 Chromatography Software for HPLC

By Franklin Lyabaya, Linda Zuvich

Shimadzu's CLASS-VP™ v5.0 Chromatography software is the newest addition to the VP Series HPLC system. CLASS-VP™ v5.0 is a Y2K compliant, true 32-bit application software package for Windows™ '95/NT 4.0. Its multi-tasking performance, combined with flexibility and data security, improve both lab productivity and data validation. This comprehensive chromatography software package includes tools for LC control, data acquisition, reprocessing, and validation, as well as custom reporting.

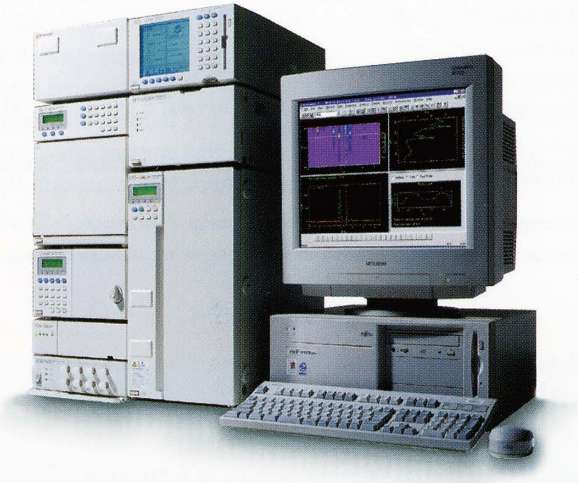

Figure 1 HPLC VP Series system with CLASS-VP v. 5.0 data station

CLASS-VP™ v5.0 Chromatography software offers:

- PC control
- Control of multiple systems with multiple methods
- Excellent data integrity
- Confidence in regulatory compliance
- Powerful PDA peak purity and library search features

Flexible, Simple Instrument Control

CLASS-VP™ v5.0 enables fully independent control of up to four complete HPLC systems with up to four detectors each, including one photodiode array detector (PDA). One instrument can be shut down and reconfigured without disrupting other running instruments. Each instrument module can acquire and process data from a variety of sources. Data is transferred digitally from Shimadzu HPLC detectores or from the optional PC-55N analog data acquisiton boards through the SCL-10AVP system controller. The SS-420 A/D board, installed into the PC and PE Nelson entefaces, is also supported.

Individual module configuration is very user-friendly. After establishing the communication param-

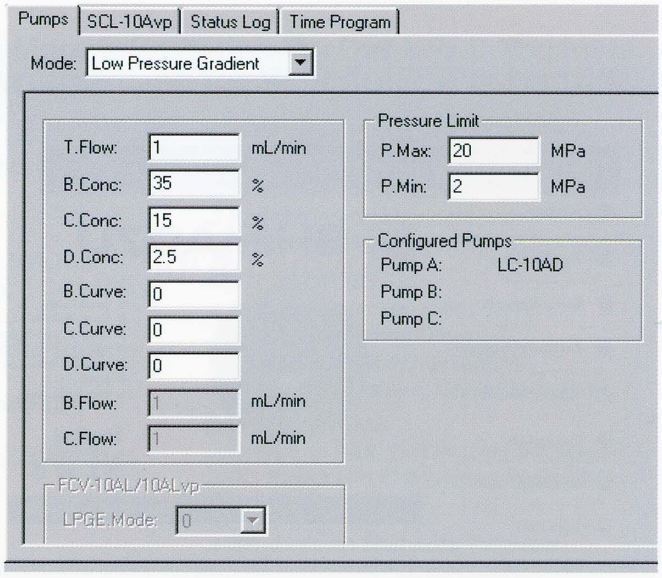

Figure 2 Pump Instrument Setup tab

Franklin Lyabaya and Linda Zuvich, Shimadzu Scientific Instruments, Inc., MD U.S.A
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This comprehensive chromatography software package includes tools for LC control, data acquisition, reprocessing, and validation, as well as custom reporting.

eters between the SCL-10AVP System Controller and the PC, the system modules are recognized and their serial numbers and ROM versions are automatically transferred to the software. Instrument Setup tabs are provided for the instrument-specific parameters of individual modules (Fig. 2).

Instrument operation through CLASS-VP™ v5.0 is intuitive. Function icons enable direct access to individual instrument modules such as pump ON/OFF, oven ON/OFF, lamp ON/OFF, and detector signal auto-zero. A complete set of integration icons is displayed as a separate toolbar for graphical chromatogram manipulation. CLASS-VP™ v5.0 also offers right-mouse button access to commonly used functions.

Each VP-series HPLC system module has special VP functions, including a maintenance log, consumable parts replacement record, and auto-diagnostics, that are downloaded to CLASS-VP™ v5.0. The system check provides a built-in maintenance log.

Excellent Data Integrity

The CLASS-VP™ v5.0 software package provides a complete set of integration, quantitation, analysis and mathematical functions for chromatography.

Comprehensive data files are embedded with all essential information (system configuration, method, data, etc.); file overwrite protection assures high data integrity. Previously acquired data can be opened with either originally calculated results or with results as they were last calculated. Long file names are accepted, and file searches can be conducted by date (day, month, or year), user ID and/or sample ID. Every data file has 4-digit date and time stamps for Y2K compliance.

Data import and export is supported in ASCII and AIA format (Level 2), so further calculations can be conducted in a spreadsheet program, and results can be shared between systems or managed by a LIMS (Lab Information Management System).

Support of the VP Series hardware confirms consistent instrument performance by enabling the direct monitoring of pressure, temperature, and flow rate parameters. Instrument condition "Status Traces" can be overlaid on the chromatograms to document operating condition uniformity from run-to-run. Several chromatograms can also be displayed as overlays, making on-line peak assignment by chromatogram comparison possible.

Confident Regulatory Compliance

The CLASS-VP™ v5.0 chromatography software supports the validation parameters of the VP Series HPLC and includes many GLP/GMP features for fail-safe operation and comprehensive compliance documentation.

Data security is ensured with passwords, multiple-user log-in, and three privilege levels (Fig.3). A

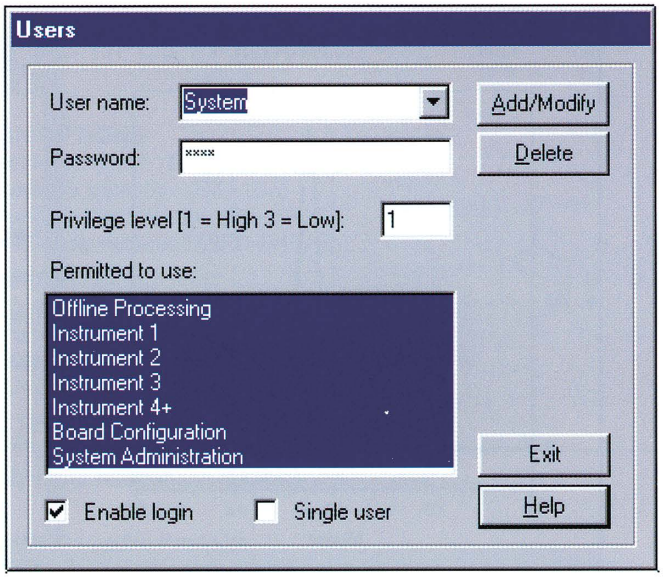

Figure 3 Each user is assigned specific privileges

complete Audit Trail can be stored for each HPLC system, method file, and data file. It includes user names, method details, and changes. Once an audit trail is enabled for a method, it cannot be disabled.

All analysis conditions, including LC system configuration, LC system control, data processing and report output, are stored as an integral part of the method. Any method modifications can be recorded in the Audit Trail for GLP/GMP compliance.

GLP/GMP compliant validation of both the instrument and the method is demonstrated through system suitability standards. Parameters evaluated include column performance, repeatability, accuracy, noise, and baseline. A pre-set Failure Action triggers

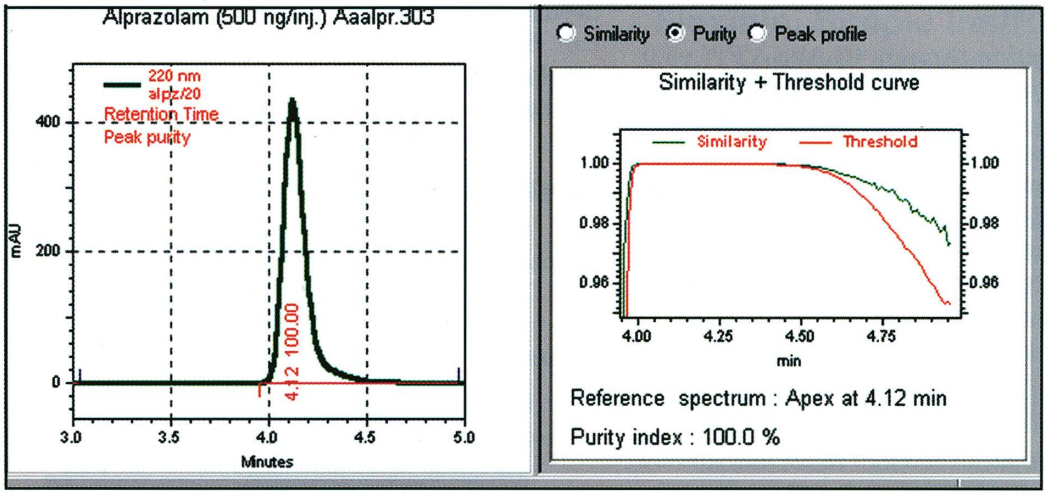

Figure 4 Purity Index Identification confirms the purity of Alprazolam

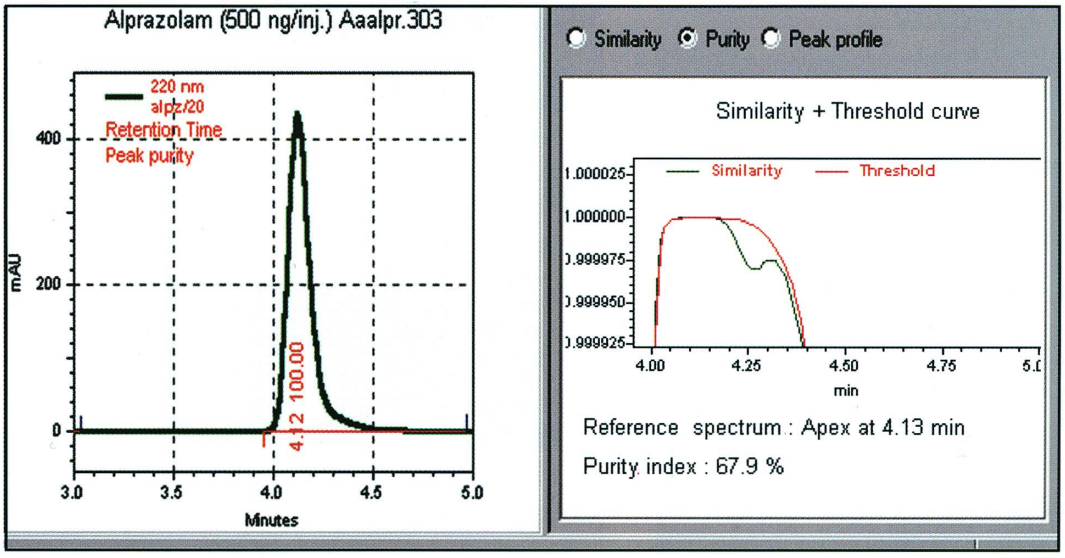

Figure 5 Purity Index Identification of 1% Triazolam in Alprazolam

the automatic re-run of standards if system suitability does not meet requirements, with re-injection or system shut-down if the required Coefficient of Variation (C.V.) is not met.

A comprehensive validation report summarizing the hardware validation functions can be printed to document compliance. In addition, a program file alteration check ensures that all installed CLASS-VP™ v5.0 program files have not been corrupted or altered.

Powerful PDA Detection Features

Special features have simplified the configuration and use of the PDA detector. Examples include a noise spectrum for the calculation of peak purity based on threshold values or similarity of spectra, similarity calculations for overlaid spectra, and simple control of the D/A interface, providing four analog output channels.

The peak table allows multi-wavelength calibration; peak names can be assigned based on retention time, spectrum and similarity threshold value.

A gallery of view options includes ratio plot, fully rotating 3-D plot, contour plot, multi-chromatograms, or any combination. All views are fully compatible with custom reporting.

The improved library structure allows filtering according to retention time, peak maxima and/or compound names. The number of candidate compounds and the number of library entries are unlimited. The library can contain a complete data set of spectra, eliminating the need for separate data sets. All prior spectra from CLASS-VP™ v4.x and EZChrom™ can be used without conversion.

Shimadzu's purity algorithm uncovers impurities even at very low levels (Figs. 4, 5). Peak purity is determined by matching each spectrum under the chromatographic peak to the spectrum at the peak apex. The percentage of spectra similar to the apex spectrum is a numerical measure of spectral homogeneity of the chromatographic peak and is reported as the Peak Purity Index (PPI).

Custom Reports

CLASS-VP™ v5.0 provides pre-formatted report templates and flexible report generation with OLE (Object Linking and Embedding) support. Select and modify a pre-defined report template, or design one. Include company logos, photographs, Word™ documents, tables, charts, spreadsheets and multiple chromatograms per page.

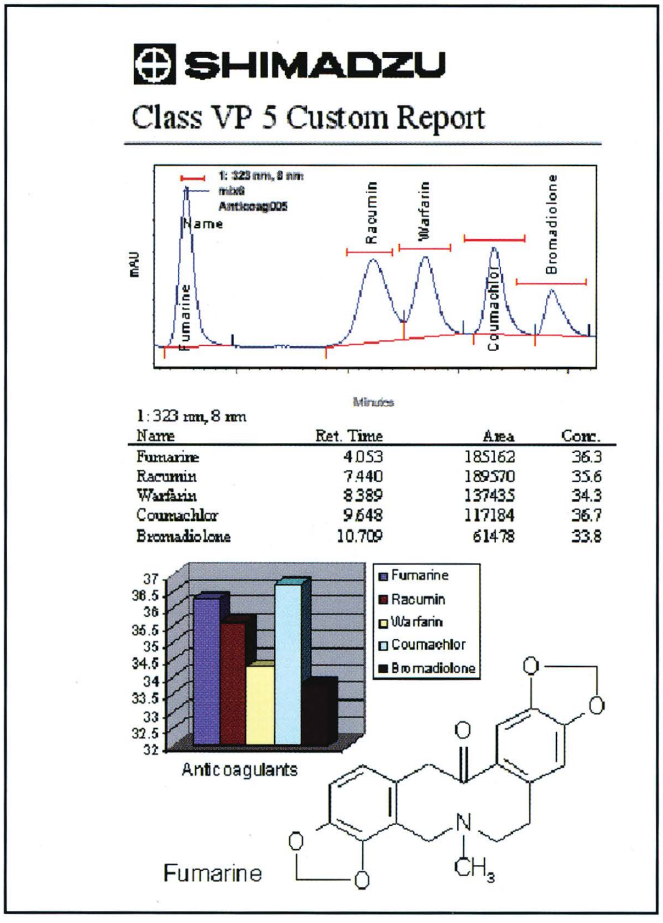

Figure 6 Example of custom report

Precision Evaluations from Microtiter Plates using the SIL-10ADvp Shimadzu Auto Injector and 3M Empore® Extractions

*by D.J. Ehersman**, L.A. Holden**, R. DeMuro*, and S.M. Wishnies**

HPLC-HPLC/MS systems have greatly advanced quantitative and qualitative bioanalysis. 3M Empore products for Solid Phase Extraction (SPE) are designed to provide analytical solutions that meet customers' needs for specificity, sensitivity, and improved turn-around times using both HPLC and HPLC/MS techniques. Advances in automated liquid handling systems, as well as improved SPE products for sample preparation, have helped to increase the efficiency of HPLC - HPLC/MS systems by redefining required elution volumes and producing eluates free of particle fines. Furthermore, these products have allowed HPLC- HPLC/MS products to achieve lower limits of detection and improved up-time for completing routine analysis. Recently, the 3M Empore technical service group undertook a quality assurance project that required automated injection from deep well plates. The 3M Empore 96-well extraction plate product was the ideal format for conducting the study; however, HPLC analysis using the existing lab auto injector would have required multiple steps and solution transfers. Consequently, a Shimadzu SIL-10ADvp auto injector and an SCL-10ADvp system controller were acquired for a thorough product evaluation as a potential solution for procedures necessitating 96-deep well injections (Fig. 1).

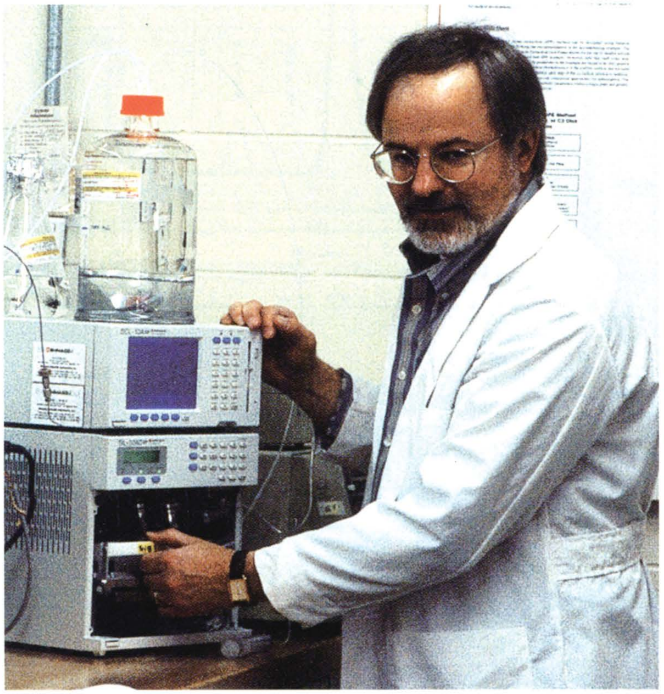

Figure 1 David Ehersman, Ph.D. and the Shimadzu SCL-10ADvp system controller and SIL-10ADvp auto injector (door removed) with the Deep Well Rack installed

Initially, the Shimadzu VP-series injector available to 3M did not support the deep well option. A collaboration between 3M and Shimadzu ensued to find a viable solution to the problem. This collaboration resulted in a fully functional deep well injection prototype (now commercially available from Shimadzu) and a modified auto injector ROM version to accommodate the deep well rack (Fig. 2).

The 3M staff worked with the SIL-10ADvp Shimadzu auto injector and the newly developed deep well rack, which accommodates the majority of standard and deep well microtiter plates. The project began with sample collection in deep well plates, followed by transferal of sufficient volumes to shallow well plates for injection. The initial study sample injections were rapidly completed using this method.

An evaluation calculating the contribution of the HPLC

N	54	55
Mean	23.3	23.53
SD	0.08	0.03
C.V.	0.34	0.12

Table 1 Repeated injections of test solution

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system (including the Shimadzu auto injector) to the total imprecision of the HPLC method was completed with repeated injections of the neat challenge (test) solution containing both theophylline and β-hydroxyethyl theophylline. The two separate series of injections yielded the statistics shown in Table 1.

Based on the superb initial precision demonstrated using the Shimadzu SIL–10ADvp auto injector, injections with the shallow well injection plate (Fig. 2) continued, followed by theophylline extractions with the Empore 96-well plates. The Shimadzu auto injector was used for all injections.

This project involved optimization of several pieces of laboratory automation to effectively complete all 1,800 extractions. The Packard Multi-PROBE® liquid handling system extracted samples from Empore 96-well SPE plates. Using the Shimadzu auto injector in conjunction with our current HPLC system, 300 extractions were completed per day, with a total chromatography run time per extraction of 3.0 minutes. The ability to inject from dual 96-well collection plates and the availability of the SIL-10ADvp short injector rinse cycle were essential for completion of this study in a timely manner. Incorporation of the Shimadzu auto injector, noted for its precision and accuracy, allowed the work to be completed quickly and efficiently. The data was promptly processed and provided to the 3M QA team for continued product support and improvements.

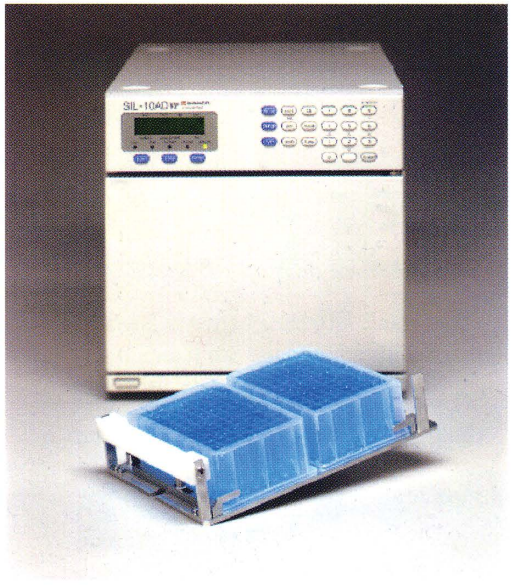


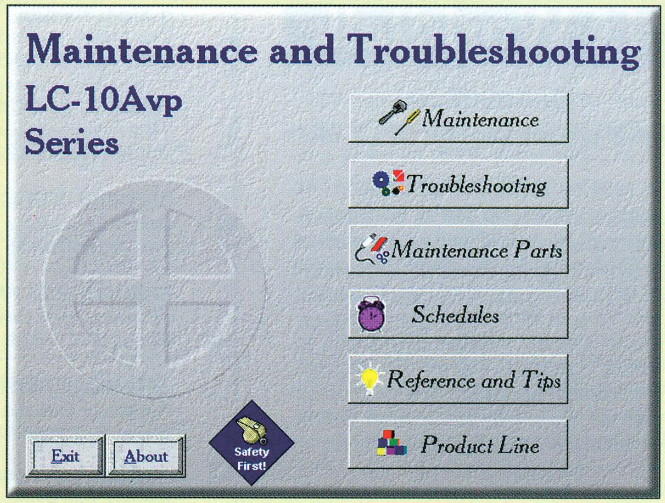
Figure 2 SIL-10ADvp auto injector with Deep Well Rack for both deep and standard 96 and 384 well plates (microtiter plates were a gift from Whatman Polyfiltronics)

Shimadzu has continued to work with the 3M Empore staff after completion of this project. Our laboratories were of the first to install and test Shimadzu's deep well rack for the VP series injector. The precision and automation of the injections provided by this prototype allowed rapid gathering and processing of information by the 3M team to ensure continuous, quality production. We continue to use the Shimadzu SIL-10ADvp system, including the fully operational deep well rack modification, today. Products such as these are essential to 3M Empore development efforts to provide new answers in the Solid Phase Extraction area of the bioanalytical market. Shimadzu's commitment to developing innovative, high-quality solutions has provided the enhanced laboratory automation and increased productivity which 3M requires to satisfy the ever-increasing demands of its customers.

Coming soon: HPLC Maintenance and Troubleshooting CD-ROM

Another tool to help you with your work will be coming soon from Shimadzu! The VP Series Maintenance and Troubleshooting CD-ROM is packed with information, procedures, and tips so you can get the most from your Shimadzu HPLC system.

The Maintenance section uses videos, narrations, and text to illustrate the most common maintenance procedures. Links to the comprehensive Parts area help you determine the part number you need for maintenance. If you are having a problem with your analysis or instrument, the searchable Troubleshooting database will help you solve it and show you how. The Schedules section helps you keep track of routine maintenance procedures and when you last performed them. Finally, the invaluable collection of HPLC tips and reference is a section you will return to again and again.

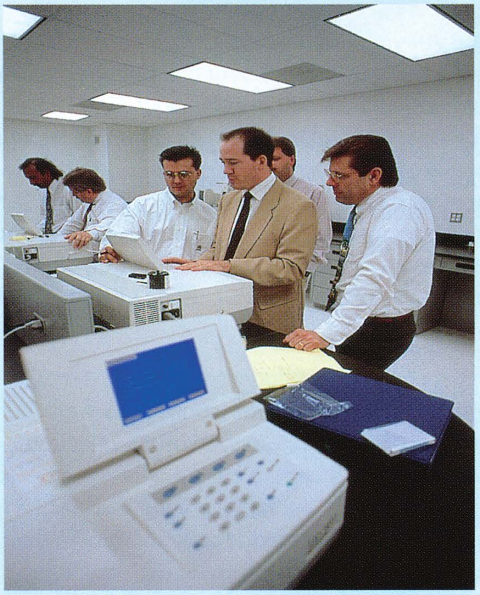


Look for details on the CD-ROM in the next issue of LC Worldtalk!

Year 2000 Challenge

The year 2000 date change is a worldwide issue that will affect businesses, customers, and individuals. Shimadzu's service and strategic planning for the year 2000 is going beyond this landmark event to assure uninterrupted business.

Year 2000 conformity means that neither performance nor functionality is affected by dates prior to, during, and after the year 2000. The year 2000 date change demands immediate, proactive attention. Older equipment may need to be updated. Shimadzu is meeting the challenge by giving our customers the high quality of service that they have come to expect. Shimadzu will:



- **Help customers understand the importance of the problem and the impact on their business.**
- **Find and correct every incidence of non-compliance in current generation products to maintain instrument integrity.**
- **Maintain operation stability to keep our customers' business functioning.**

Shimadzu has identified internally and maintains documents on all affected software and hardware made by Shimadzu. We have also manufactured instrumentation and software compliant with the year 2000 date change. For more information, please visit the year 2000 area of our website, http://www.shimadzu.com.

LCWorldtalk

international

Shimadzu's newletter for HPLC users

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