C190-E097

Spring 2006 / International Edition

SHIMADZU
Solutions for Science
since 1875

LC World Talk

SHIMADZU'S NEWSLETTER FOR THE HPLC GLOBAL COMMUNITY

Fast LC Utilizing Prominence

There's No Other HPLC System Like It In The World. The World's First Web Control, Fastest Sample Injection, And Highest Detection Sensitivity Performance.

Fast LC Utilizing Prominence

There's No Other HPLC System Like It In The World. The World's First Web Control, Fastest Sample Injection, And Highest Detection Sensitivity Performance. With the Prominence series of HPLC components, you get enhanced specifications for practical, real-world HPLC, and the performance and flexibility necessary for any application. The SIL-20A autosampler utilizes a unique needle treatment and inert contact materials to virtually eliminate carryover while increasing your LC sample throughput with high-speed injection cycles.

Fast LC: You Want High Throughput Not High Pressure

Today's demand for increased efficiency in the analytical business has highlighted the important technical challenge of providing faster separation in HPLC. The direct approach is to increase the mobile phase flow rate (linear velocity); however, increasing the mobile phase flow rate in the widely used 5µm particle diameter packed column diminishes column efficiency.

In order to further enhance performance, there are multiple possible approaches but the following two seem the most promising:

1. Use of smaller particle size packing material
2. Separation under elevated temperature

The common goal of the two approaches is to reduce the Height Equivalent to Theoretical Plate (HETP) and, consequently, to increase the column performance per a given column length in a high flow-rate region. There are several factors to consider in both approaches.

1. Use of smaller particle size packing material

The technological approach of decreasing particle size in the packing material is one way to enable more rapid separation. As shown in *Figure 1 (see following page)*, the HETP remains small even under a higher flow-rate range if smaller particle size packing material is used.

The plots indicate that smaller particle size packing material makes it possible to achieve high column efficiency under a high flow rate, enabling good separation quickly. There are several columns packed with sub-2µm packing material for faster separation.

However, this approach is problematic because the currently marketed hardware will be subjected to a greater load due to increased column pressure (which is inversely proportional to the square of the particle diameter), requiring limited column length. *Figure 2* shows the relationship between the flow rates and column pressures with different sized packing materials.

The back pressure drastically increases as the particle size decreases. This makes it difficult to use the smaller particle columns under an optimum (higher) flow-rate range or a long column to get high resolution. To address this, specialized instruments with improved pressure resistance for high-speed separation are now on the market. However, these specialized instruments still have certain limitations in the pressure tolerance and sacrifice injection accuracy and detection sensitivity, precluding their use in normal analysis. The question arises: is it really necessary or beneficial to use sub-2µm packing material for increasing the speed when the back pressure increases so much?

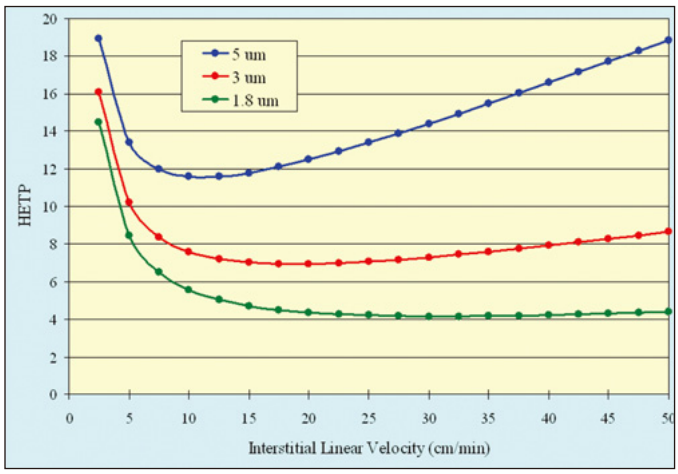


Figure 1: van Deemter plots with different particle size packing material columns

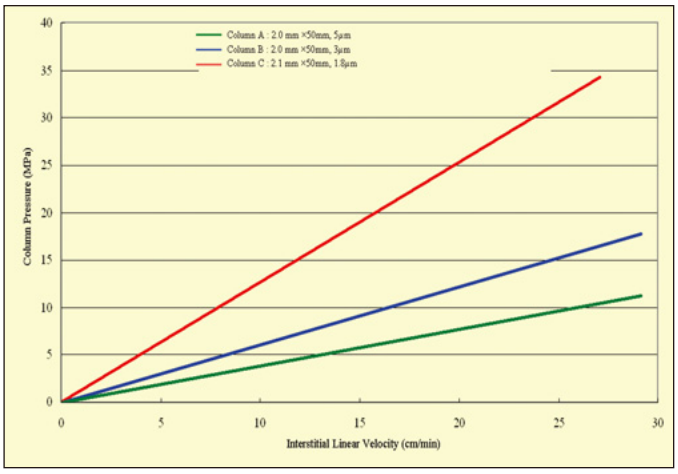


Figure 2: Relationship between linear velocity and column pressure

Shimadzu has been working on addressing this issue and now proposes the solution that makes it possible for any user to enjoy high speed and high resolution simultaneously with their existing instrumentation. The solution is to use a column packed with very uniform 2.2µm packing material. As seen in ***Figure 3 A–C***, the column pressure (red line) drastically increases as the particle size decreases, while the performance improvement (blue line) is very limited at a smaller particle size range. Stated differently, the performance with a 2.2µm column is not very different from that of a 1.8µm column but the expected back pressure is less than 2/3. By using Shimadzu's new Shim-pack XR-ODS, the back pressure is less than half of the commercially available 1.8µm column. This means it is no longer necessary to give up either performance or ease of use; you can get the highest performance without sacrificing operability.

Additionally, for some cases smaller particle columns are not suitable for separating closely eluted peak pairs. If resolution is very critical, the natural strategy is to use a longer column to improve resolution. With a small particle column, it may be impossible to use a longer column because the back pressure may exceed the allowed operating pressure. As a result, it is necessary to choose a lower flow rate or a shorter column to reduce the back pressure since, as mentioned above, column back pressure is inversely proportional to the square of the particle diameter.

Figure 4 shows the relationship between the particle size and needed separation time to separate two compounds, which takes 30 minutes for a 25cm column with 5µm packing material to separate them at Rs=1.5. Obviously, a smaller particle column can shorten the needed time until the particle size reaches 2.2µm. However, because of the higher pressure of the sub-2µm column, the column length is limited at the fixed pressure (60MPa). Consequently, the actual theoretical plate number gets lower and the resolution poorer at the given mobile phase conditions. It is necessary to weaken the solvent strength to completely separate the compounds, which causes a longer retention time.

Figure 5 indicates that critical separation was achieved in a shorter time with the Shimadzu 2.2µm column than with a commercial 1.8µm column since it was possible to use a longer column to get higher plate numbers without sacrificing the analysis time. The compounds are D-phenylalanine derivative and L-phenylalanine derivative. The mobile phases were the mixture of 100 mmol/L (potassium) phosphate <pH6.8> / acetonitrile (upper: 77/23, lower:84/16, v/v), the column temperature was 50°C and the UV detection was performed at 350 nm.

Table 1 shows that a longer column with rather big particles (i.e. 5µm) should be used if you want to get the highest theoretical plate numbers under the given column pressure limit. The time needed to get the performance is much longer so a balance exists between the time and resolution. 2.2µm columns seem to be optimal for balancing the time reduction and high resolution at the highest level.

Particle size (µm)	Opt. Linear velocity (cm/min)	Minimum HETP (µm)	Column length to reach 60MPa (mm)	Achievable plate number
1.8	31.11	4.16	104	25,048
2.2	25.46	5.08	190	37,418
3.0	18.67	6.93	482	69,579
5.0	11.20	11.55	2,232	193,275

Table 1: Relationship between particle sizes and achievable plate numbers

Figure 6 shows an example of time reduction using a 2.2µm column. The analysis time was reduced by 6 times without sacrificing resolution or using too much high pressure.

As shown, the selection of the particle size is critical to get the optimum results and smaller particles do not necessarily offer better results. High pressure is simply a consequence of particles that are too small. Shimadzu believes it would be more useful if the same or better separation with a shorter run time can be achieved with lower back pressure.

2. Separation under elevated temperature

There is increasing interest in elevated temperature LC since better and faster separation can be simultaneously obtained by using higher temperature, which has the effect of accelerating diffusion of the species while decreasing resistance to flow in the column.

It is a characteristic of columns that efficiency is highest at a particular mobile phase flow rate, but at flow rates less than and greater than that flow rate, efficiency decreases (HETP increases). This relationship is typically expressed using the following van Deemter equation:

$$H = A\,dP + B/\nu + C\,dP^2\,\nu$$

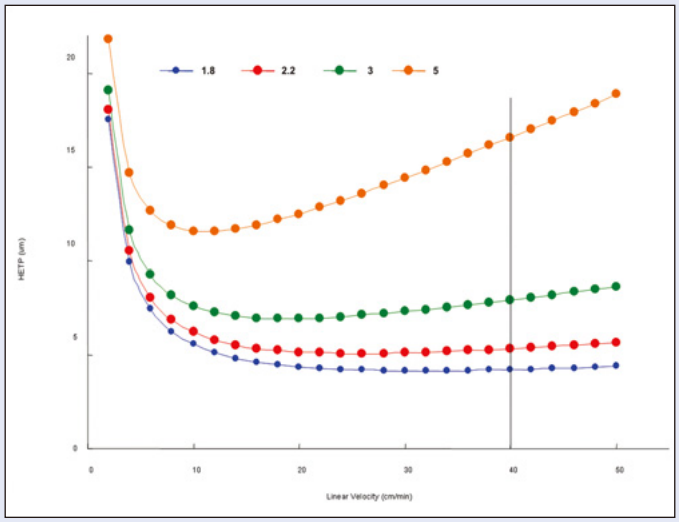
H is the HETP (column length required to obtain 1 theoretical plate - the smaller this value, the greater the efficiency), dP is the packing particle diameter, and ν is the mobile phase linear velocity. The applicability of smaller packing material with a micro-sphere packed column for performing higher-speed separation is attributed to the lack of deterioration in efficiency at high flow rates through two additional terms in the equation. Both factor A (eddy diffusion) and factor C (coefficient of mass transfer) decrease as temperature increases. ***Figure 7*** shows van Deemter plots at different temperatures. Lower HETP is obtained under a higher temperature especially at a higher flow-rate range.

Additionally, separation at high temperature has an effect similar to that of decreasing particle diameter. Increasing temperature improves the diffusion aspect of the C term in the van Deemter equation, allowing for a flatter curve at higher flow rates. The resistance to column flow is inversely proportional to temperature, such that the column pressure at 80°C is about 40% lower than that at 40°C.

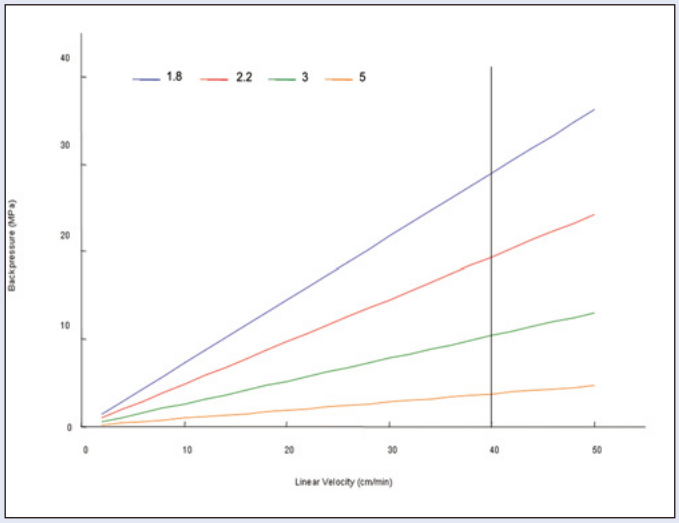
This highlights the importance of temperature as a factor in accelerating separation by demonstrating that analysis time can be effectively shortened by raising the column temperature while increasing the mobile phase flow rate.

As long as the packing material is stable under the elevated temperature, this technique is quite powerful to get higher resolution and higher speed without making many changes to the system configuration. ***Figure 8*** shows an example of the effect of elevated temperature LC. The ZirChrom-PBD can be used at a higher temperature and shows much shorter separation time.

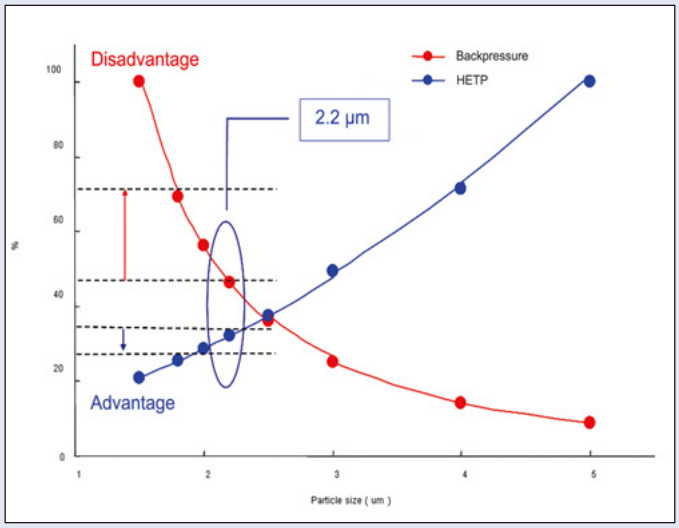
Figure 3A—C: Examining the considerations for obtaining high speed and high resolution while maintaining operability. High performance, with low backpressure, is achieved by using 2.2µm particles



A.



B.



C.

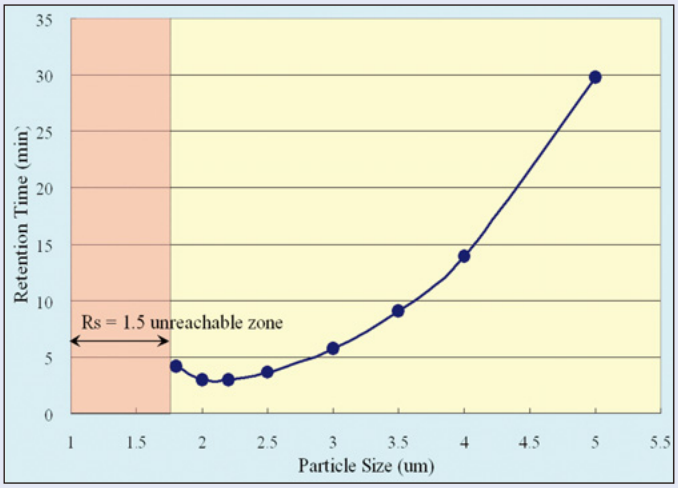


Figure 4: Time necessary to achieve Rs=1.5 for a certain compound pair

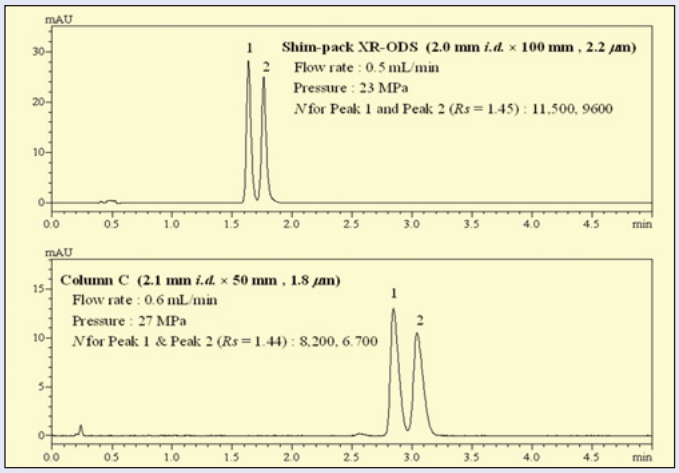


Figure 5: Critical separation shown

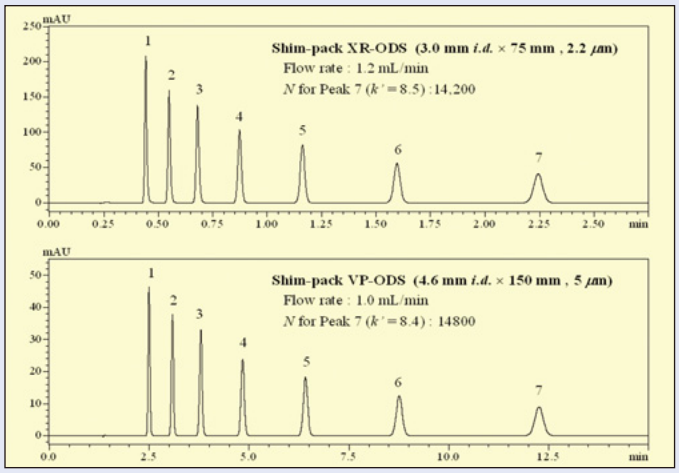


Figure 6: An example of time reduction using a 2.2μm column. Chromatographic conditions – mobile phase: water/acetonitrile (3/7, v/v), temperature: 40°C, detection: absorbance at 245 nm. Peaks; 1: actophenone, 2: propiophenone, 3: butyrophenone, 4: balenophenone, 5: hexanophenone, 6: heptanophenone, 7: octanophenone.

Another example is shown in ***Figure 9***. In this example, the combination of elevated temperature LC at 125°C and an ultra-fast autosampler made it possible to repeat isocratic runs in about a 30-second cycle without adopting an overlap injection technique.

To make elevated temperature LC successful, the rapid heating capability is critical since the temperature gradient in the column caused peak shape distortion. Prominence supports temperature control up to 85°C using powerful forced-air column ovens. The ovens are powerful enough to eliminate a dead volume caused by a big preheater, which is necessary for block-heating-type ovens. If temperature higher than 85°C is needed, the CRB-6A, which can go up to 150°C, can be used.

3. Prominence—most suitable HPLC for Fast LC applications

Prominence Series HPLC was introduced in the United States in 2005. Shimadzu added a number of enhancements while maintaining full backward compatibility to the LC-VP series. The key enhancements include the following:

1. Very high pump resolution (3nL)
2. Ultra-fast injection speed (10 seconds)
3. Stable and reliable operation at 85°C (up to 150°C with CRB-6A)

Those enhancements made Prominence the most suitable HPLC for Fast LC applications. ***Figure 10*** shows retention time and peak area reproducibility at extremely fast gradient elution normally used for high-speed separation. As mixer capacity must also be reduced, achieving high retention

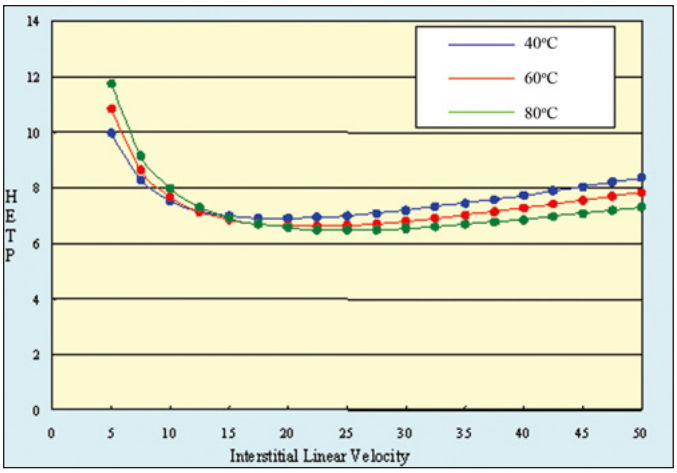


Figure 7: van Deemter plots at different temperatures. Chromatographic Conditions – Column: Shim-pack C18 (3 mm i.d. × 100 mm, 3μm); Mobile Phase: Water/Acetonitrile (40/60, v/v); Detection: 245 nm, Sample: Alkylphenones.

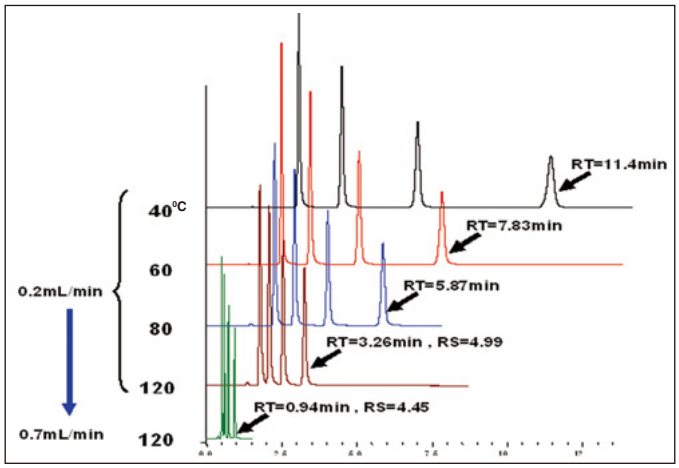


Figure 8: Fast separation using the elevated temperature LC. Chromatographic Conditions – Column: ZirChrom-PBD; MP: 40% acetonitrile; Analytes: valerophenone, hexaphenone, heptaphenone, octaphenone.

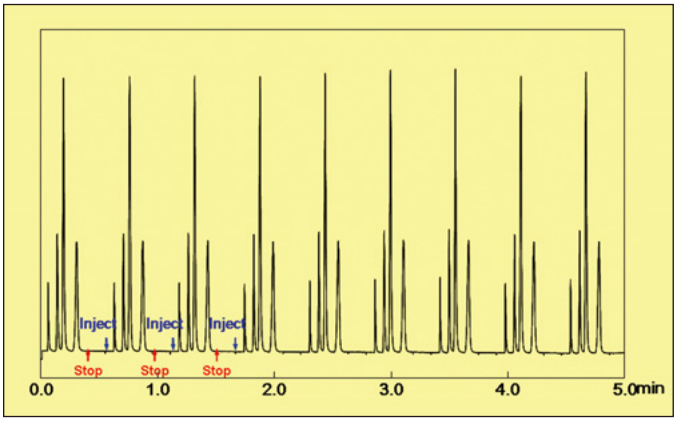


Figure 9: Fast isocratic analyses using the elevated temperature LC. Chromatographic Conditions – Column: ZirChrom-PBD (3mmx50mm); Mobile phase: 30% acetonitrile; Flow rate: 3mL/min; Temp: 125°C (CRB-6A); Analytes: uracil, toluene, naphthalene, biphenyl.

time reproducibility is more difficult than in normal analysis. Prominence displays excellent pumping performance with its micro stroke delivery and 3nL flow-rate resolution, showing high retention time reproducibility even with a 100µL capacity mixer. Also, unlike specialized high-speed separation units, the needle-in-flow-path design autosampler can be used in high-speed and standard analysis, enabling excellent injection accuracy and very low carryover suppression.

The performance of the system was further pushed in *Figure 11* where a 23-second cycle time was achieved with gradient runs with no overlap injections. The first three runs were completed within 1 minute and showed good reproducibility. The ultra-fast, no carryover SIL-20A autosampler along with the ultra-high performance LC-20AB binary pump made this happen. The autosampler injected a sample every 9 seconds and the binary pump changed the concentration of the strong solvent from 60% to 95% in 2.4 seconds. The high-performance CTO-20AC column oven elevated the mobile phase temperature to 80°C in a short time to minimize the back pressure under the high flow rate and ensure a good peak shape. It should be noted that the experiments were performed under 35MPa (5,000psi). This clearly proved high pressure is not needed to get high throughput. High pressure is just a consequence of using an unnecessarily small particle column.

4. Fast LC applications

For Prominence, Fast LC is simply one of the ways this versatile HPLC system can be utilized. Here are a couple of applications obtained using the 2.2µm columns.

Figure 12 shows a separation of PTC-amino acids. The complete separation of 17 amino acids' derivatives was complete in less than 4 minutes, which is 6 times faster than conventional LC with a 5µm column and 2 times faster than high-speed LC with a 3µm column.

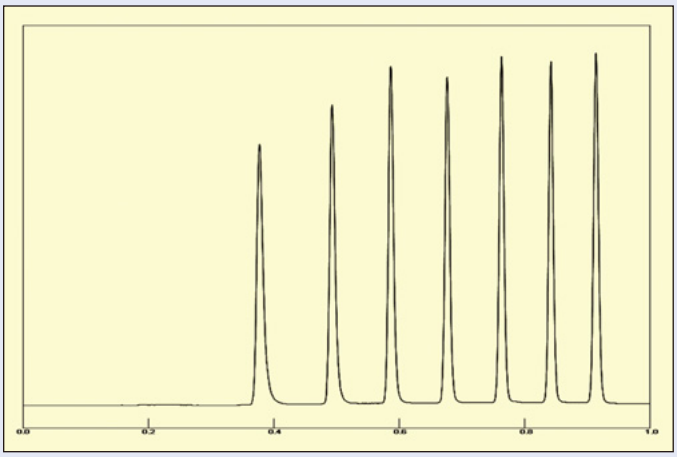


Figure 10: Retention time and peak area reproducibility at very fast gradient runs. Chromatographic conditions – Column: Shim-pack XR-ODS (3 mm×50 mm); Mobile phase: A: water/B: acetonitrile, 0min (50%B) → 0.55min (95%B) → 0.70min (95%B); Flow rate: 1.5 mL/min; Temperature: 40°C; Detection: UV 245 nm; Sample: alkylphenone mixture.

Compounds	Retention Time		Peak Area	
	Average	%RSD	Average	%RSD
Acetophenone	0.377	0.076	49018	0.193
Propiophenone	0.493	0.072	47260	0.167
Butyrophenone	0.587	0.070	49199	0.055
Valerophenone	0.677	0.065	45277	0.138
Hexanophenone	0.763	0.082	46613	0.138
Heptanophenone	0.842	0.087	43975	0.205
Octanophenone	0.914	0.080	48978	0.200

Table 2: Retention time and peak area reproducibility at fast gradient runs.

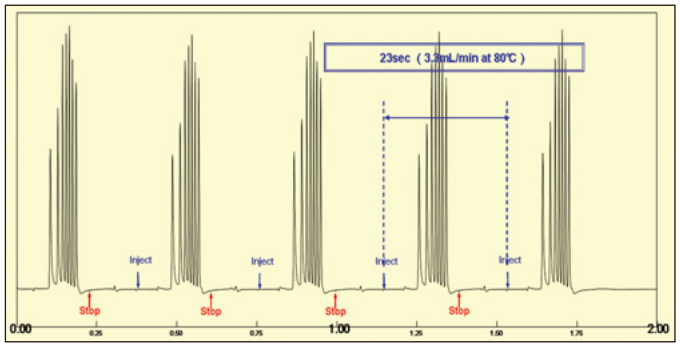


Figure 11: Ultra-short cycle time gradient runs. Chromatographic conditions – Mobile phase: A: water, B: acetonitrile, 0min(60% B) → 0.04min (95%B) → 0.09min (95%B), 3.3mL/min; Temperature: 80°C; Detection: absorbance at 245 nm, 0.23min stop; Peaks: acetophenone, propiophenone, butyrophenone, Valerophenone, hexanophenone, heptanophenone, octanophenone.

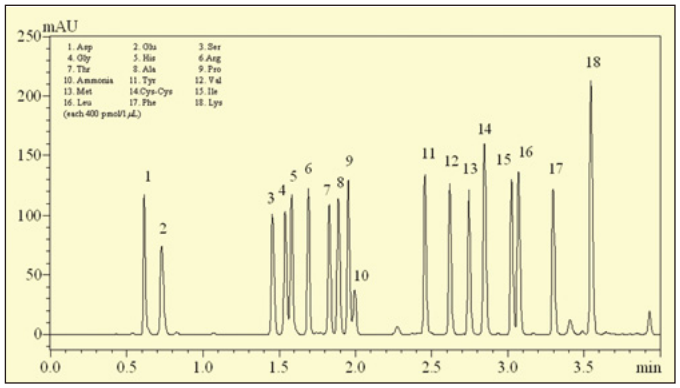


Figure 12: Analysis of PTC-amino acids standard solution (400pmol/uL each). Chromatographic conditions – Column: Shim-pack XR-ODS (2.2µm, 3.0 mm i.d. × 75 mm); Mobile phase: 10 mmol/L (potassium)phosphate <pH 7.0> for A and acetonitrile for B (A/B: 95/5 for 0.3 min then 95/5 to 60/40 in 3.4 min); Flow rate: 1.2 mL/min; Temperature: 40°C: Detection: absorbance at 254 nm.

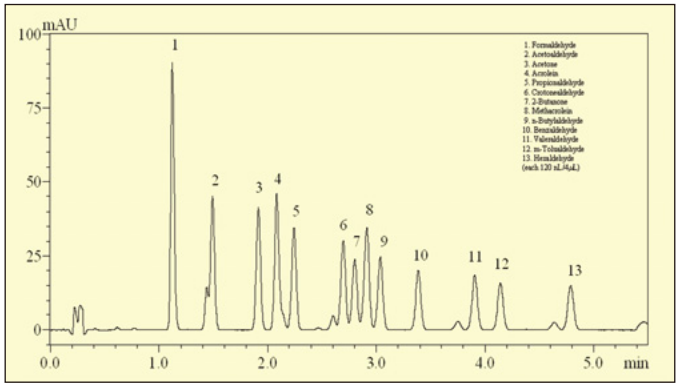


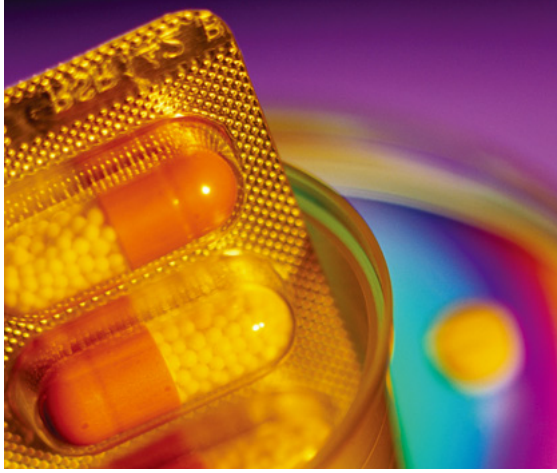
Figure 13: Analysis of 2,4-DNP-aldehydes/ketones standard solution (120nmol/4uL each). Chromatographic conditions – Column: Shim-pack XR-ODS (2.2µm, 3.0 mm i.d. × 75 mm); Mobile phase: water/THF (8/2,v/v) for A and acetonitrile for B (A/B: 8/2 to 5/5 in 5 min); Flow rate: 1.2 mL/min; Temperature: 50°C; Detection: absorbance at 360 nm.

"The combination of the columns with a Fast LC-ready Prominence system makes a really Fast LC system. The versatility of Prominence is not sacrificed: users can use a column switching system and/or a dual-column gradient system along with the Fast LC configuration."

Figure 13 shows another example of Fast LC applications. 2,4-DNP-aldehydes and ketones were separated in less than 5 minutes, which is 6 times faster than conventional LC with a 5µm column and 3 times faster than high-speed LC with a 3µm column.

5. Conclusion

As shown, the goal of Fast LC is not high pressure but high throughput, meaning how many samples can be analyzed per day or per hour. In order to achieve high throughput, both the run time of one chromatogram AND the cycle time should be shortened. The solution from Shimadzu addresses the dilemma of using very small particle size columns by providing unique 2.2µm packing material columns, which generate less than half the back pressure compared to commercially available 1.8µm packing material columns. The combination of the columns with a Fast LC-ready Prominence system makes a really Fast LC system. Even in this configuration, the versatility of Prominence is not sacrificed: users can use a column switching system and/or a dual-column gradient system along with the Fast LC configuration. This is the real solution for users in terms of performance, reliability, ease of use, and affordability.



LCMS-IT-TOF

Analysis of Amoxicillin

The Shimadzu LCMS-IT-TOF incorporates the higher MS stages available with an ion trap and the high resolution and mass accuracy of a time-of-flight (TOF) mass spectrometer, leading to the only mass spectrometer in the field that allows for excellent mass accuracy for both parent and fragment ions (up to MS^{10}).

The LCMS-IT-TOF utilizes patented ion introduction techniques for both the ion trap and TOF region of the mass spectrometer. Compressed ion injection was developed to efficiently accumulate and compress the ions prior to introduction into the ion trap (Figure 1). Within the ion trap, all ions are simultaneously ejected into the TOF (ballistic ion extraction) instead of the traditional mode of scanning ions out of the trap region. The simultaneous extraction of the ions out of the trap region leads to lower spatial distributions for the ions as they enter the TOF region, thus leading to better resolution. Resolution is also enhanced through the use of argon gas for cooling the ions (Figure 2). Argon, a larger molecule than helium, which is traditionally used, has a higher dampening efficiency and does not lead to fragmentation. Ions entering the trap within the LCMS-IT-TOF have a lower energy than ions found within a traditional ion trap mass spectrometer experiment.

Another key difference between the LCMS-IT-TOF and its competitors is the fast polarity switching (100 msec) found uniquely with this instrument, allowing for high mass accuracy maintained in both modes of analysis for one single run.

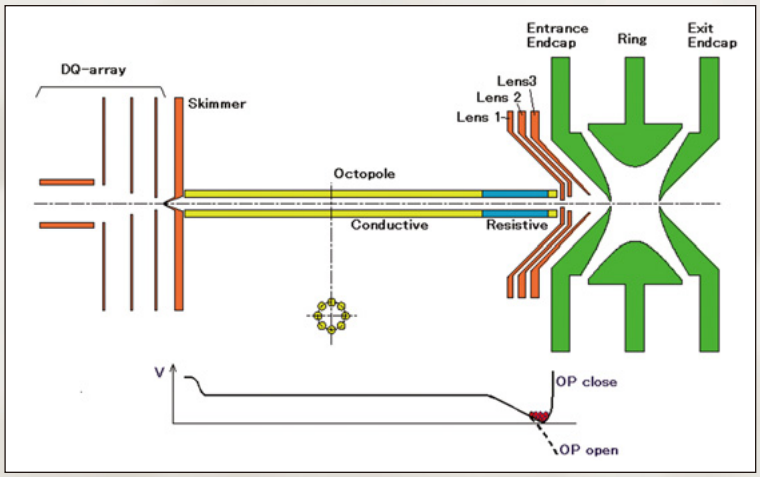


Figure 1: Schematic of the LCMS-IT-TOF MS configuration & ion introduction optics (Compressed Ion Injection) - Ions are focused in an octopole partially coated with a resistive material close to the trap. An electric potential gradient along the ion beam axis forms in the resistive region. When a voltage is applied to the electrode adjacent to the octopole, a 'potential well' forms in which ions are accumulated. The focused 'packet' of ions is rapidly pulsed into the ion trap.

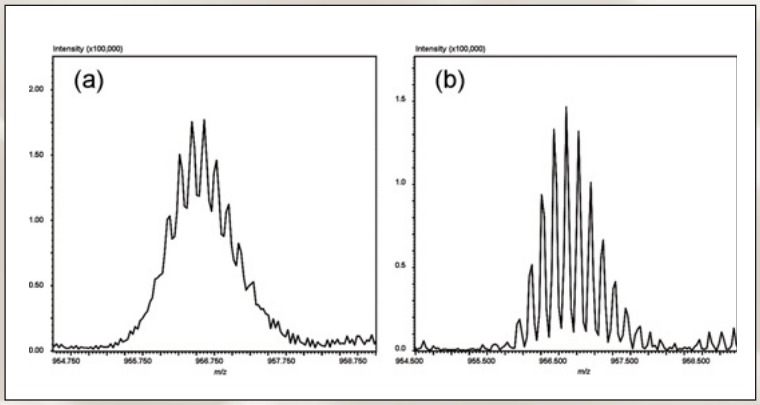


Figure 2: Resolution - Mass spectra of bovine insulin (m/z 956, $[M+6H]^{6+}$) with (a) He cooling and (b) Ar cooling. Argon is used as the cooling gas, but unlike traditional ion trap analyzers, mass resolution is not degraded. The higher dampening efficiency of Ar better localizes trapped ions and the initial space and energy distribution for the TOF analyzer are reduced, leading to higher mass resolution and accuracy.

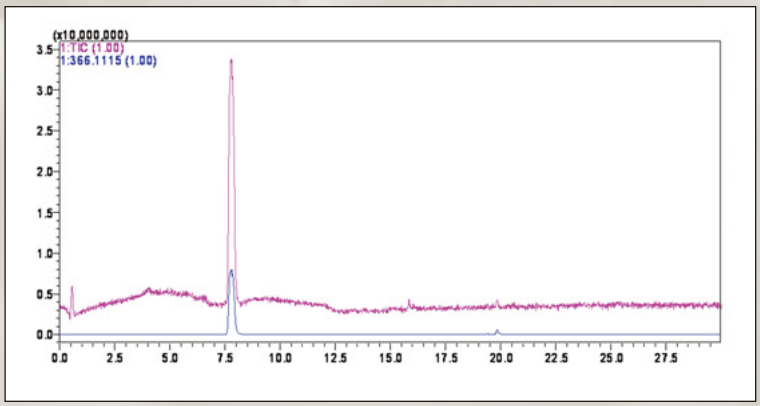


Figure 3: TIC and extracted mass chromatogram for amoxicillin.

Amoxicillin

Amoxicillin is a drug belonging to a class of compounds known as ß-lactam antibiotics. Amoxicillin, a member of the penicillin family, is used most often to treat a number of bacterial infections including *H. influenzae, N. gonorrhoea, E. coli, Pneumococci, Streptococci*, and some strains of *Staphylococci*. It is thought that these penicillin-derived compounds work to stop the bacteria from multiplying by inhibiting its cell wall synthesis. Confirming these antibiotics in animal-related food sources is important to human health since many people can be sensitive to these drugs and/or develop resistance to the effectiveness of such antibiotics with overexposure.

A number of publications have focused on the identification and quantitation of amoxicillin and other members of the ß-lactam antibiotics family. Groups interested in confirming the presence of these antibiotics both in animal tissues and bovine milk have used ion trap mass spectrometry for its fragmentation ability.[1,2] More recently, the ability to quantitate such drugs in animal tissues has also been published utilizing triple quadrupole mass spectrometers.[3,4] In terms of qualitatively confirming the presence of such antibiotics, the Shimadzu LCMS-IT-TOF is able to generate characteristic fragmentation spectra as well as high mass accuracy information useful for confirming structure. In this experiment, the mass accuracy of amoxicillin and its fragments is showcased along with the MS^n ability of the LCMS-IT-TOF.

Experimental Methods

Amoxicillin and LC-MS grade formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Burdick and Jackson HPLC water and acetonitrile were purchased through VWR

(Bridgeport, NJ). LC-MS analysis was performed using the Shimadzu Prominence Series LC coupled to the LCMS-IT-TOF. Prominence Series components included two LC-20AD pumps, SIL-20A Autosampler, and a CBM-20A System Controller. The column used for reversed-phase LC analysis was a Shimadzu Shim-pack VP-ODS packed with 4.6 µm particles (4.6 x 150 mm). The instrument was controlled through LCMSsolution, and data analysis was performed using the same software, but utilizing the LCMS Postrun Analysis feature.

The LC analysis consisted of the following conditions: Mobile phase A: 95% H_2O (0.1% Formic Acid) + 5% ACN; Mobile phase B: 95% ACN + 5% H_2O (0.1% Formic Acid); Flow rate: 0.300 mL/min; Gradient: 0 min 0% B, 15 min 25% B, 23 min 50% B, 25 min 50% B, 26 min 5% B. Stop time: 30 min. A 0.5 µL injection volume was used.

The LCMS-IT-TOF was operated under the following conditions: ESI in positive mode; drying gas: 1.5 L/min; CDL temperature: 200°C; interface temperature: 200°C; ion accumulation time: 50 msec; MS^1 scan range: 100 – 1000 m/z, MS^2 scan range: 101 – 400 m/z, MS^3 scan range: 97 – 400 m/z; CID parameters: Energy – 100%, collision gas – 100%, time – 30 msec.

Results and Discussion

One of the preferential cleavages for amoxicillin is the loss of NH_3 observed in both the MS and the MS^2 spectra. Another important characteristic cleavage among ß-lactam antibiotics is also the opening of the ß-lactam ring (160 m/z) (Figure 5). In terms of screening, differentiating the ß-lactam antibiotics containing an amino group from other antibiotics within the ß-lactam family is possible using the fragments at 349 and 208 m/z.[1]

The LCMS-IT-TOF does not rely on an internal standard to give high mass accuracy data. Simply performing a shortened version of the autotuning function built into LCMSsolution allows one to routinely achieve mass accuracy measurements well below 10 ppm. Similar studies that show the quality of mass accuracy data that can be acquired using an Agilent LC/MSD TOF utilize a dual-sprayer source for the simultaneous measurement of a reference ion (internal standard), an approach utilized by a number of mass spectrometry manufacturers. Comparing data published by Nägele et. al. on the measurement of amoxicillin using the LC/MSD TOF, mass measurements acquired on the LCMS-IT-TOF are comparable if not better *(see Table 1)*.

A number of publications have focused on the identification and quantitation of amoxicillin and other members of the ß-lactam antibiotics family. Groups interested in confirming the presence of these antibiotics both in animal tissues and bovine milk have used ion trap mass spectrometry for its fragmentation ability.

Formula	Calculated mass $(M+H)^+$	Measured mass $(M+H)^+$	Mass accuracy (ppm)
$C_{16}H_{19}N_3O_5S$	366.1118	366.1114	1.1
$C_{16}H_{16}N_2O_5S$	349.0853	349.0841	3.4
$C_6H_9NO_2S$	160.0427	160.0425	1.3

Table 1: Mass accuracy values for amoxicillin and its CID fragments using the LCMS-IT-TOF. Note: Analysis performed without an internal standard.

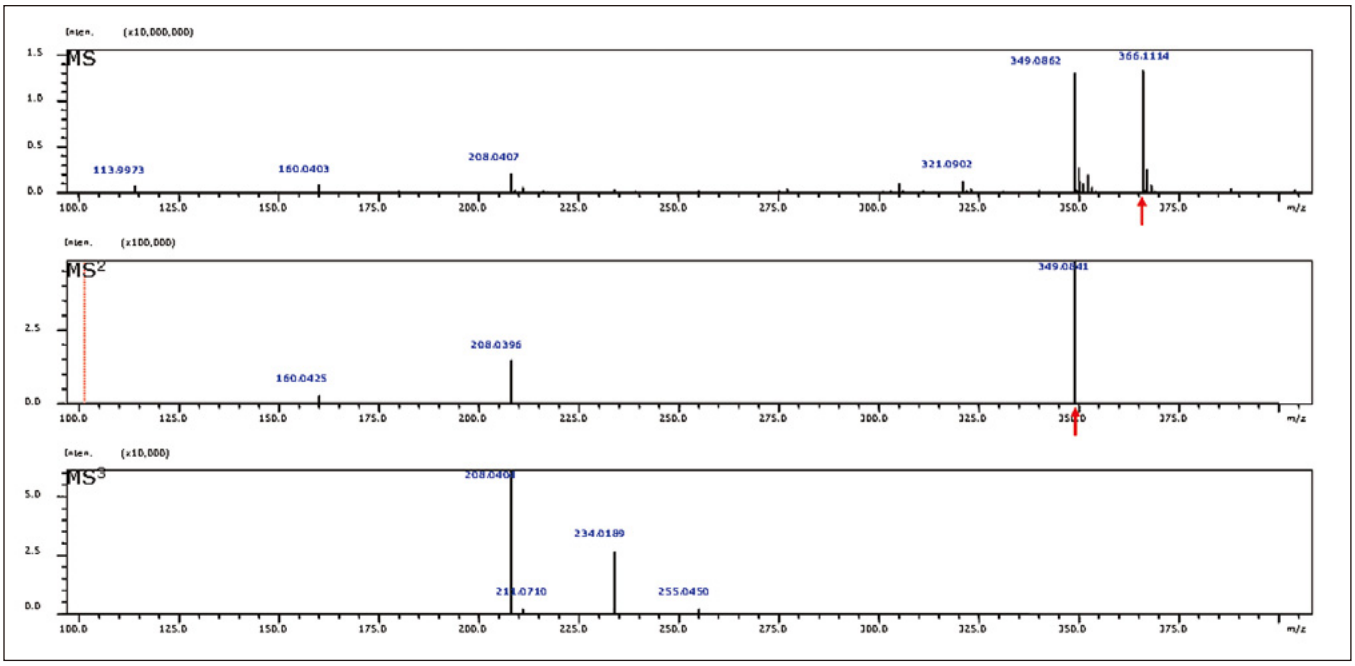


Figure 4: Analysis of amoxicillin [$(M+H)^+_{thr}$ = 366.1118] on the LCMS-IT-TOF - Precursor for MS^2 - 366.1114 m/z; Precursor for MS^3 - 349.0841 m/z (indicated by red arrows).

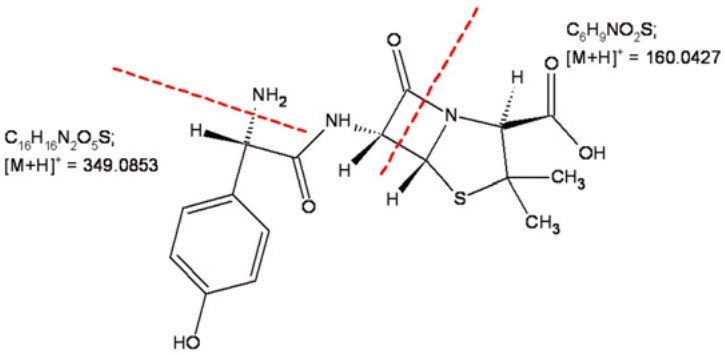


Figure 5: Structure of Amoxicillin, $C_{16}H_{19}N_3O_5S$; $[M+H]^+$ = 366.1118 with cleavages and the corresponding fragment masses.

The mass accuracy for the same fragments reported by Nägele et al. was found to be 1.4, 1.7, and 5.0 ppm for the same ions when accounting for the electron (an error found in the published report lists the mass accuracy at 0.18, 0.23, and 1.71 respectively). Important to note, these mass accuracy measurements were acquired with the use of the reference ion mass as discussed earlier.[5]

Conclusions

Amoxicillin was successfully separated and detected under gradient conditions using a Shimadzu Prominence series LC coupled to the LCMS-IT-TOF. Data acquired on the LCMS-IT-TOF allows for MS^n and excellent mass accuracy within one experiment. Mass accuracy data obtained from the LCMS-IT-TOF is comparable to data reported by other vendors requiring the use of an internal standard or a dual-sprayer configuration.

References

1. Heller DN, Ngoh MA. *Rapid Commun. Mass Spectrom.* 1998; 12: 2031-2040.
2. Fagerquist CK, Lightfield AR. *Rapid Commun. Mass Spectrom.* 2003; 17: 660-671.
3. Baere SD, Cherlet M, Baert K, De Backer P. *Anal Chem.* 2002; 74: 1393-1401.
4. Fagerquist CK, Lightfield AR, Lehotay SJ. *Anal Chem.* 2005; 77: 1473-1482.
5. Näegele, E., Moritz, R. Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MS^n and accurate mass determination by ESI TOF. *J. Am Soc Mass Spectrom* 2005, 16: 1670-1676.

LCGC magazine presents an interactive, streaming video Webcast

Exploiting HPLC Techniques and Innovations to Enhance LCMS Performance and Productivity

The HPLC front-end: More than just an inlet

The adoption and utilization of MS detection for HPLC analysis has grown tremendously in the past several years. What was once an esoteric and problematic technique has almost become, in the case of a single quadrupole, a routine HPLC detection method. With the advent of better interfaces and more advanced software control, the use of even the high-end instruments has become commonplace for many applications. Truthfully, without the abilities of advanced MS routines, many of the benefits provided by the pharmaceutical and other industries could not have been realized. This discussion will focus on ways to improve the performance and productivity of the user's MS based on HPLC inlet considerations.

Learn Critical Techniques that Will Drive Efficiency and Optimization

- Design aspects to consider when choosing an autosampler
- Rinsing "Dos and Don'ts" in pursuit of zero carryover
- Autosampler effect on LC-MS/MS system sensitivity and dynamic range
- Realizing the best quantitation limits
- Increased throughput using multiple front-end systems on a single MS/MS

Join David Walsh and a panel of distinguished experts for an exciting, educational web event featuring diverse viewpoints and suggestions on enhancing LCMS performance and productivity. This complimentary Webcast includes live Q&A and download of reference materials.

For more information or to register visit LCGCmag.com/HPLCTech

Presented by:

LCGC north america
solutions for separation scientists

Sponsored by:

SHIMADZU

Live: Thursday, May 18, 2006 @ 2:00 PM EST
Duration: 1 Hour

Register Free On-line
LCGCmag.com/HPLCTech

Interact with the Experts During Live Q&A

David Walsh
Editor in Chief
LCGC Magazine
Moderator

PANELISTS

John Dolan, PhD
Partner
LC Resources, Inc.

Mauro Aiello, PhD
Application Chemist
Applied Biosystems/ MDS Sciex

Mike Larson
Senior Staff Scientist
Covance Laboratories

[CUSTOMER CASE STUDY]

SHIMADZU

Opens New Mass Spectrometry/ Biotech Applications Laboratory

New lab supports research and development, and provides expert support resources

THE LCMS-IT-TOF ANALYSIS OF AMOXICILLIN APPLICATION was produced at Shimadzu Scientific Instruments' new Mass Spectrometry/Biotech Applications laboratory at its headquarters in Maryland. Part of Shimadzu's Global Customer Support Centers, the lab is intended to enhance U.S. customer support, serve as the central U.S. facility for developing methods and systems, and conduct R&D in collaboration with academic and industrial organizations.

Opened in fall 2005, scientists in the lab are exposed to advances in equipment as soon as they are available, and can give feedback and commentary based on their real-world experiences. The new lab features the following instruments:

- High-Throughput Prominence HPLC Series
- Award winning LCMS-IT-TOF
- LCMS-2010EV
- ChIP-1000 Chemical Printer
- AccuSpot MALDI Plate Spotter
- Axima-CFR+ MALDI-TOF Mass Spectrometer
- Xcise Integrated Gel-excision Processor

One of Shimadzu's main goals in building the Mass Spectrometry/Biotech Applications laboratory is to actively support research by creating application notes, performing consultations, conducting in-depth demonstration/ training sessions, and analyzing customer samples. The five applications chemists at the lab, assisted by technical support personnel in the field, comprise an expert resource for researchers tackling difficult application questions and provide a wide range of customer support options.

"The goal of this lab is to assist researchers by making available the personnel and the equipment to address specialized LCMS/biotech applications," said Masayuki Nishimura, Ph.D., Manager of the Mass Spectrometry/ Biotech Applications laboratory. "We welcome inquiries for tours and demonstrations."

Online help: anytime, anywhere

Interactive LC Virtual Advisor

The LC Virtual Advisor includes a maintenance section that provides animated, easy-to-follow maintenance procedures.

The web-based, interactive LC Virtual Advisor offers 24-hour interactive support for the Prominence HPLC Series. With 24-hour, password-protected access, users can custom-configure virtual systems to match their current or dream HPLC system configurations. The user can then access animated easy-to-follow maintenance procedures specific for their configured Prominence system. Users can also access the Troubleshooting section, which leads them to problem-solving procedures. A newer Advanced Troubleshooting module allows users to diagnose and correct many of the problems encountered with HPLC applications, using a step-by step logical sequence of questions designed to troubleshoot chromatography issues.

The LC Virtual Advisor offers added convenience and learning opportunity, as the web site includes a reference/education section with a glossary of HPLC-related terms, and an ever-increasing list of other reference applications.

To learn more and register, please visit **www.shimadzu.com/lc_virtualadvisor**

Article Submission
LC WorldTalk invites you to submit your articles and papers. Please send a brief abstract (100-250 words) for consideration. Abstracts may be submitted to LCWorldTalk@shimadzu.com (please put "Abstract" in the subject line). You may also send abstracts to:

Shimadzu Scientific Instruments
Attn: LC WorldTalk
7102 Riverwood Drive
Columbia, MD 21046
Fax 410-381-1222

If accepted for publication, a representative from Shimadzu will contact you. Comments and suggestions, or requests to receive LC WorldTalk may be e-mailed to **LCWorldTalk@shimadzu.com.** LC WorldTalk is available online at **www.ssi.shimadzu.com/LCWorldTalk**

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
Cable Add.:SHIMADZU TOKYO

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.
7102 Riverwood Drive, Columbia, Maryland 21046, U.S.A.
Phone: 1(410)381-1227 Fax. 1(410)381-1222 Toll Free: 1(800)477-1227

SHIMADZU EUROPA GmbH
Albert-Hahn-Strasse 6-10, 47269 Duisburg, F.R. Germany Phone: 49(203)7687-0 Fax. 49(203)766625

SHIMADZU (ASIA PACIFIC) PTE LTD.
16 Science Park Drive #01-01 Singapore Science Park, Singapore 118227, Republic of Singapore
Phone: 65-6778-6280 Fax. 65-6779-2935

SHIMADZU SCIENTIFIC INSTRUMENTS (OCEANIA) PTY. LTD.
Units F, 10-16 South Street Rydalmere N.S.W. 2116, Australia
Phone: 61(2)9684-4200 Fax. 61(2)9684-4055

SHIMADZU DO BRASIL COMÉRCIO LTDA.
Avenida Marquês de São Vicente, 1771. Barra Funda CEP : 01139-003-São Paulo-SP, Brasil
Phone: (55)11-3611-1688 Fax. (55)11-3611-2209

SHIMADZU (HONG KONG) LIMITED
Suite 1028 Ocean Center, Harbour City, Tsim Sha Tsui, Kowloon HONG KONG
Phone: (852)2375-4979 Fax. (852)2199-7438

SHIMADZU INTERNATIONAL TRADING (SHANGHAI) Co., LTD. SHANGHAI OFFICE
24th Floor, Shanghai Xin Hualian Building, No.755 Huaihai Zhong Lu, Shanghai, China
Phone: 86-21-6472-8442 Fax. 86-21-6472-8648

Overseas Offices
Istanbul, Moscow

URL http://www.shimadzu.com

Printed in Japan 3295-05603-50ANS