Lab Manager MAGAZINE
Run Your Lab Like a Business

INSIGHTS
LAB TECHNOLOGY BUYER'S REPORT

INSIGHTS ON MASS SPECTROMETRY SYSTEMS

A LAB MANAGER MAGAZINE
TECHNOLOGY BUYER'S REPORT

SUPPLEMENT TO LAB MANAGER MAGAZINE

April 2012
VOLUME 2
NUMBER 2

CONTENTS

INSIGHTS ON MASS SPECTROMETRY SYSTEMS

All articles, except "Ask the Expert", by Contributing Editor, **James Netterwald**, Ph.D., MT(ASCP)

James Netterwald, Ph.D., MT (ASCP), can be reached at james.netterwald@yahoo.com or by phone at (973) 588-3532.

INSIGHTS
LAB TECHNOLOGY BUYER'S REPORT

THE LIFETIME OF LC-MS IS, AND WILL BE, CONSTANTLY EVOLVING

LC/MS Purification System | Gilson www.gilson.com

With lightning-fast speed, LC-MS systems have rapidly evolved into today's highly functional tools. But where did they begin? "When LC-MS instruments first came out 15 to 20 years ago, the main issue was just getting them to work," says Bob Classon, LC-MS business development manager, Shimadzu Scientific Instruments (Columbia, MD). Back then, the early models were difficult to use and lacked the ionization sources needed, especially for small-molecule pharmaceuticals. "Once people learned how to make them work, the next step was making them reliable—making them work for weeks or months without having to do a lot of maintenance," says Classon. About seven years ago, the trend in MS switched more to a focus on sensitivity, prompting the development of a number of LC-MS systems with large increases in sensitivity. "The trend now is more toward convenience and reliability, which means a number of systems were created that require less maintenance and can handle a wider variety of samples and sample matrices without difficulty," says Classon.

One of the latest trends is a move toward improving operator convenience by reducing the training requirements needed to use LC-MS. "Right now we have a lot of people using them who have no analytical chemistry, mass spectrometry, or HPLC background," says Classon. "Users might range from biomedical people or synthesis chemists to food chemists." LC-MS users don't want to know the technology or the physics of how it works. They want results, and fast. So the robustness and the ability to handle a dirty sample or dirty matrix, or compatibility with fast HPLC or UPLC, are of great interest to current end users. Recently introduced instruments are more robust than products from just five years ago, and the newest instruments are getting faster to be more compatible with UPLC and high-throughput requirements. Instruments now have to be able to perform quantitation on chromatographic peaks that are less than one second wide and have to be able to perform quantitative and qualitative analysis on fast peaks in a single one-minute run. Much of this is driven by clinical labs and CROs where the lab that runs the most samples per day makes the most profit. Even as pharmaceutical companies are producing fewer NDAs, they are trying to get better drugs into clinical trials faster. They don't want the LC-MS analysis portion to slow down getting a drug to market.

NEW COMPOUNDS; LIGHTNING SPEED; COMPLETE SOLUTIONS

The trend moving forward will probably place somewhat less emphasis on small molecule analysis and more on biotherapeutics, protein-based compounds, and biosimilars. Here the selectivity is just as important as the sensitivity. Samples involving proteomic workflows generally incorporate many steps such as concentration, fractionation, digestion, and other types of cleanup that were never designed to be put together. In order to speed up analysis of these types of samples and to reduce cost per sample, there will be more emphasis on the systems to automate these steps so users gain not only speed but also quantitative transfer from step to step and much greater reproducibility. Systems are already available to allow for injection of whole-plasma samples for fast analysis of proteins. These systems use antibody binding and immobilized enzymes to perform analysis of targeted proteins for research applications such as finding markers for cancer and other diseases. One such system, the Perfinity Workstation, can select target proteins from a biological sample, perform a tryptic digest, and produce a peptide map, all automatically. If you were looking for the metabolism of a protein-based drug or a low-abundance protein biomarker, this type of analysis would complement the LC-MS significantly by allowing a biological sample to be processed, digested to peptides in under 10 minutes, and turned into a full peptide map in 30 to 60 minutes. In short, this type of system makes the LC-MS work better. The conventional approach to this is that a 24-hour analysis is much more expensive and is not as reproducible for quantitation.

"Another trend is the life science industry's continued push for more efficiency and productivity," says Lester Taylor, Ph.D., LC-MS product marketing, Agilent Technologies (Santa Clara, CA). "To that end, with mass spectrometry moving into other fields outside pharmaceutical or research fields, there is the need for ease of use in providing access of this data to nonspecialists." The push for efficiency is not only a push for greater ease of use but also for automation, particularly in front-end sample handling using high-throughput robotics.

▼*LC-MS System | Flexar SQ 300*
PerkinElmer | www.perkinelmer.com

> "The push for efficiency is not only a push for greater ease of use but also for automation."

A further trend in LC-MS is for vendors to provide complete solution kits, which include the mass spectrometer, the analytical methods, the HPLC conditions, and the mass spectrometry conditions, as well as a database

containing reference spectral information. "That allows the user to run the samples, get the data back, and screen it against its library of spectra," says Taylor.

Another trend is the development of software tools to allow the inexpert LC-MS user to analyze samples and get streamlined summary reports.

NEW APPLICATIONS; PERSONALIZATION

According to Sal Iacono, vice president and general manager of mass spectrometry for PerkinElmer (Waltham, MA), one of the major trends in the LC-MS market is greater use of the instrument in preclinical research and food testing, and there is an increase in the ease of use of these systems. "Over the last few years, we have seen more multifunctional researchers. Having the time to learn every software package, every nuance of it, is rapidly disappearing. The capability to make one or two mouse clicks to generate a result is one of the major trends in the marketplace and is likely to accelerate further," says Iacono.

Besides the traditional LC-MS users in basic life science research and pharmaceuticals, there are an increasing number of users in clinical diagnostics and in food safety who are using the technology for routine applications. "These users don't want just a mass spectrum. They want yes-or-no answers," says Steve Smith, senior director and mass spectrometry product manager, Waters Corporation (Milford, MA). "These are critical applications where the mass spec result could make the difference between life and death or making and losing millions of dollars."

▼ *Triple Quad Mass Spectrometer | LCMS-8030 | Shimadzu*
www.ssi.shimadzu.com

Another trend in LC-MS is to personalize the operation of the instrument. "If you have six scientists in the lab, then you have two ways to personalize those instruments: you could either buy a new instrument for each user, or you could schedule time for each individual to operate an instrument," says Iacono. "What we've designed is a separation probe that is about the size of a writing pen. Each individual can be assigned a personalized separation probe. In this way, when a scientist is ready to use the instrument in a scheduled manner or via open access … the individual simply walks up to the instrument and places the separation probe onto the mass spectrometer with the click of one button." A column can be preconfigured right down to the chromatographic separation. "When the scientist is finished with the analysis, the user disconnects the LC and walks away with that personalized application probe," says Iacono. These novel solutions will reduce the cost of operation to the laboratory by maximizing efficiency, optimizing usage time, and ultimately requiring smaller numbers of instruments and solvents.

In summary, there is a wide variety of trends in the LC-MS market. Present and future end users should keep abreast of these trends in order to effectively make the most informed purchasing decisions.

KEEP YOUR LC-MS HAPPY AND HEALTHY BY FOLLOWING THESE REQUIREMENTS

Like much of the instrumentation used in life science laboratories nowadays, LC-MS instruments have become faster, more accurate, and easier to use over the course of their evolution. These days, there are specific minimum requirements that every laboratory should keep in mind before purchasing an LC-MS instrument.

EDUCATIONAL REQUIREMENTS

The educational requirements for LC-MS operators vary from one company to another. Generally LC-MS operators are not required to be university-educated or analytical chemists. "Quite often LC-MS operators are technicians who have a high school education along with some kind of vocational training," says Steve Smith, senior director and mass spectrometry product manager, Waters Corporation (Milford, MA). Ideally, the user will perform the following duties on the instrument: sample loading, cleaning and maintenance, and generating the MS report. Most MS systems boast ease of use as one of their important features, which allows users who are not trained Ph.D.-level mass spectrometrists access to this powerful tool.

LC/GC/MS & Elevating UHPLC Benches
IonBench | www.ionbench.com

Operating an LC-MS instrument does require a lot of training, but the amount of training depends on the operational function to be performed. For very routine applications, a minimum of a high school diploma plus training is acceptable. When the operator has advanced responsibilities, a minimum of a college degree plus training is required. To perform research using a mass spectrometer, a Ph.D. is required. Many companies, such as Thermo Fisher Scientific, offer courses to train new and experienced users in their customers' labs. "We try to minimize the training by making the software as easy to use as possible, as well as by making the tuning and calibration of instruments automated," says Ian Jardine, Ph.D., vice president and chief technology officer for life sciences and mass spectrometry for Thermo Fisher Scientific (San Jose, CA).

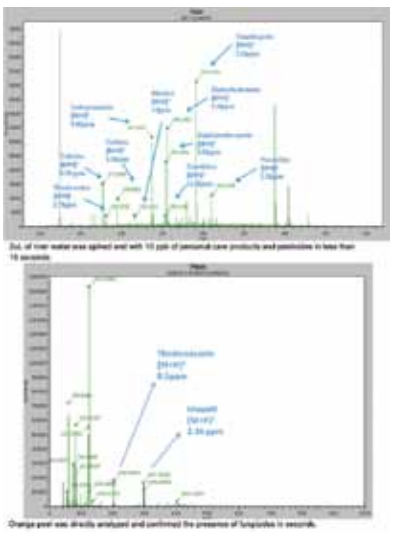
CONSUMABLES: SOLVENT USE, COLUMNS

The main consumable for any LC-MS system is the solvent, and these systems require a continuous supply of solvents. "As mass spectrometry becomes more sensitive, the demand for high-quality solvents increases," says Smith. LC-MS systems require regular maintenance, such as tuning and calibration, and the solvent or solvents may be needed for these purposes as well. "However, the level of required maintenance is becoming less and less as these systems are becoming more and more reliable," says Smith.

Solvent usage is necessary for effective chromatographic separation. Solvent usage is also a key aspect in LC mass spectrometry operations. End users are optimizing their sample separations with large amounts of acetonitrile or methanol. On multiple instruments over long periods of time, they are spending exorbitant amounts of money for use and disposal of these solvents. "PerkinElmer is reducing these costs by providing novel technologies that reduce solvent usage by at least a factor of 10, if not 20 or 30, while generating rapid, high-quality results. We accomplish this with our proprietary LC-MS interfaces; our unique, personalized separation probes; and even with our new AxION DSA (Direct Sample Analysis) system, which eliminates the need for chromatography to introduce samples to the mass spectrometer," says Sal Iacono, vice president and general manager of mass spectrometry for PerkinElmer (Waltham, MA).

▲ *LC-MS-MS Certified Vials | TruView™ Waters | www.waters.com*

▼ *Analyses Using the Direct Sample Analysis System AxION | PerkinElmer | www.perkinelmer.com*



"As mass spectrometry becomes more sensitive, the demand for high-quality solvents increases."

Chromatography columns are also an important consumable for LC-MS systems. Chromatography columns cost approximately $300 on average. "So if you are quantifying analytes in a sample every five minutes, then you might have to replace the column every week or

every couple of weeks," says Jardine. This is a high-throughput example. However, if the user is performing very low-throughput applications, then not as much of the annual budget is spent on replacement columns.

OVERALL REQUIREMENTS

In terms of overall operations, throughput—that is, the number of samples that can be processed per unit of time—is an important feature that one should consider before purchasing an LC-MS system. Since throughput is an important factor in determining the cost per sample, the level of throughput influences the cost of ownership and therefore would influence the model or platform that is purchased. Disposable sample cards are used to insert the sample into the LC-MS system for processing.

In terms of maintenance costs for LC-MS, the system owner can expect to spend about 5 to 10 percent of the initial cost of the system annually. For example, for $500,000 worth of LC-MS equipment, the annual maintenance costs would be a minimum of $25,000 to $50,000. Although this might be cost-prohibitive for some labs, the return on investment for LC-MS is high enough that these maintenance costs become negligible.

In summary, educational requirements, consumables, throughput, and maintenance costs are absolutely crucial to the decision to purchase an LC-MS system. As this instrumentation evolves, it is likely that the requirements will become greater in number and complexity.

➤ *Compact Mass Spectrometer | expression™ Advion | www.expressioncms.com*

IT'S ALL ABOUT THE ROI

In today's economic climate, it is necessary to ensure that all purchased equipment will provide a return on investment. There are numerous factors that impact an ROI calculation for mass spectrometers. One of these factors is the throughput of the instrument. "Throughput is also much higher today. Customers easily could spend $100,000 to $200,000 on a mass spectrometer. And the argument for spending that much is always increased productivity," says Ian Jardine, Ph.D., vice president and chief technology officer for life sciences and mass spectrometry for Thermo Fisher Scientific (San Jose, CA).

When trying to make a business case for purchasing an LC-MS system, cost of ownership and cost per sample are important criteria. As mentioned earlier, throughput affects the cost per sample. "And cost per sample may be based on the volume of samples to be analyzed on an annual basis or the competitive pressures (if it's a commercial lab) to do that analysis and compete on quality, turnaround time, and price. So throughput is important there," says Lester Taylor, Ph.D., LC-MS product marketing, Agilent Technologies (Santa Clara, CA).

ROI CALCULATIONS

Waters Corporation works with their customers on return of investment calculations for LC-MS systems. "Our applications specialists in the field routinely work with lab managers to work out the return on investment for our systems. Sometimes the customer is looking to move from a traditional HPLC system to a UPLC and LC-MS system, and our field experts would help their customers figure out things such as solvent consumption and throughput," says Steve Smith, senior director and mass spectrometry product manager, Waters Corporation (Milford, MA). "The customer might be a service provider that is charging their customers a certain amount per analysis, so they would want to purchase an LC-MS system in which the cost per analysis is low."

"When trying to make a business case for purchasing an LC-MS system, cost of ownership and cost per sample are important criteria."

Other companies are doing the same. "The team at PerkinElmer creates scenarios to stimulate and simplify return on investment questions every day. We put ourselves in the technicians' and the lab managers' shoes constantly," says Sal Iacono, vice president and general manager of mass spectrometry for PerkinElmer (Waltham, MA). "All our system solutions are focused on significantly lower initial operational costs, lower maintenance costs, and, most importantly, wonderfully simple means of generating results."

▲*Quadrupole-based PTR-MS System*
PTR-QMS 500 Series | IONICON | www.ionicon.com

▲*High-Throughput Mass Spectrometry System*
RapidFire 300 | Agilent | www.agilent.com

"This return on investment equation also takes into account the cost of consumables—for example, LC columns, solvents, etc.—so that for several high-throughput assays, the Agilent RapidFire/MS system becomes a very cost-effective approach and more than justifies the initial capital cost of the instrument," adds Taylor.

"All our system solutions are focused on significantly lower initial operational costs, lower maintenance costs, and, most importantly, wonderfully simple means of generating results."

Finally, a comment by Lester Taylor at Agilent cemented the concept. Says Taylor: "I've been doing return on investment calculations to help labs make the decision for the purchase of an instrument that costs several hundred thousand dollars. The return on investment would take into account the total outlay. In other words, it would look at the total number of samples in the lab, total number of instruments, number of samples per instrument, and how much revenue that instrument is effectively generating in a year. And that gives you sort of a return on investment approach to look at it."

COMMENTARY FROM A WATERS CUSTOMER

OUR USER:

Rachel Garlish, Ph.D., *Principal Scientist,* UCB, Slough, UK

Q: Please tell me how your company uses mass spectrometry.

A: UCB uses mass spectrometry in several different ways. Our lab in the Department of Physical and Analytical Sciences, New Medicines, uses it for a broad range of applications. At the very high end, we use LC-MS to perform experiments involving hydrogen deuterium exchange for epitope mapping on proteins. For this application, we use the nanoACQUITY UPLC system and SYNAPT G2 mass spectrometer from Waters Corporation. We have ancillary accessories for this system, including the HDX manager box. The SYNAPT G2 instrument allows us to separate molecules by size and shape as well as by mass with ion mobility MS, and with its associated software we can efficiently analyze the data.

Q: What caused you to select those particular MS systems for your work?

A: I believe Waters sells the only commercially available instrument that can perform hydrogen deuterium exchange. It is a complete solution for this application. We can use this instrument from the robotic liquid handling right through to data handling. The most important aspects of this mass spectrometer are its robustness and ease of use. The latter is important because we want our scientists to get up and running quickly. There are several things about this instrument that enable it to perform the hydrogen deuterium exchange. The first is definitely the automation, or robotic side, of liquid handling. The nanoACQUITY UPLC allows for very efficient chromatographic separation. Also, all our experiments must be performed at 0°C, so the HDX manager box allows us to cool the sample and do chromatography under those conditions.

"At the very high end, we use LC-MS to perform experiments involving hydrogen deuterium exchange for epitope mapping on proteins."

HOW TO BEST UTILIZE MASS SPECTROMETRY FOR DIVERSE APPLICATIONS

OUR EXPERT:

Stephen Barnes, Ph.D.,
Professor of Pharmacology & Toxicology,
University of Alabama, Birmingham, AL

Stephen Barnes, Ph.D., Professor of Pharmacology & Toxicology and Director of the Targeted Metabolomics and Proteomics Laboratory at the University of Alabama at Birmingham (UAB), talks about the changes taking place in the field of mass spectrometry (MS) as it migrates from the research lab to a clinical environment, for analysis of small molecules as well as large molecules like proteins and lipids. He also discusses some of the challenges facing MS users, particularly with data analysis and storage, when working with large amounts of MS data.

Q: Can you tell us about the types of MS instruments you have and what you use them for?

A: We cover a very wide range of applications: quantitative pathway analysis; quantification of phosphosites; oxidation and other post-translational modifications of proteins; lipidomics and individual lipids (e.g., prostanoids and isoprostanes); and other small molecules (polyphenols, particularly isoflavonoids). We don't do much in the way of discovery proteomics. We tend to focus on measuring particular compounds that people already know about before they come to us or studying a particular pathway that has been found to be undergoing a lot of changes. What we then do is set up quantitative assays for all the proteins in that pathway. We are also working in an interesting frontier that I have named "metabolo-peptidomics" or "peptide-metabolomics." Proteins are not just proteins but are sources of peptides, which have different properties than the parent protein, and we have been studying lots of small peptides involved in interesting biology. In our lab we have three hybrid triple quadrupole/linear ion trap MS systems from AB Sciex, including the Triple-TOF (time-of-fight) 5600 and an older matrix-assisted laser desorption/ionization (MALDI)-TOF instrument. We have a specific set of uses for each instrument, and we find that very useful.

Q: Have you seen a shift in the use of MS in recent years?

A: It's coming back to a more precise form of MS. I have done MS since the 1960s, and the interfaces that we take for granted today were not there then. But what we could do was measure the mass of ions very accurately. In the late 1980s with the advent of the modern MS like MALDI, and the electrospray process, the instruments had very high resolution and people were able to apply MS to a ton of things they had never been able to do before. We certainly rode that wave and acquired our first triple quadrupole instrument in 1992. But by many accounts it was a lousy mass spectrometer since it didn't have good mass accuracy, which is what a mass spec should be. So now the field has moved back to high-accuracy MS and this is absolutely necessary in proteomics if we have to turn the corner to get to actual clinical usage. Triple quads have been the mainstay of quantitative analysis for the past 18-plus years and they may still have their place in low-complexity scenarios. But for complex biological matrices, they are no longer enough. The proteome is denser than people seem to understand and if a mass spectrometer is to be used for clinically meaningful analysis, the mass analyzer for the compound's fragment ions has to have high mass accuracy and high mass resolution—a quadrupole analyzer or an ion trap can't provide that. With newer instruments, like the AB Sciex 5600, instead of collecting one fragment at a time,

the instrument collects all the ions with a mass accuracy of about 2 to 4 ppm, and then the odds that you are measuring the right peptide are considerably enhanced. Personally, I think this is a game changer and I suspect that for quantitative proteomics, the day of the triple quadrupole is over.

Q: How should people go about choosing the right MS instrument for their needs?

A: I did an experiment several years ago with a colleague, Dr. Jim Mobley, looking at some protein samples on an ion trap LTQ and on a Q-trap. Both experiments were done on the same day, so there was no day effect. We then put the data into the same informatics format and analyzed it with a single search program. My colleague found the same number of proteins that we found, but only 25 percent of the proteins were in common. We have done other experiments since and found that this represents the bias of the instrument. The Q-trap is biased toward measuring peptides with an m/z below 1000 and the ion trap does best for peptides with an m/z above 1000. We got a center grant to perform platform analysis and I worked with a group of statisticians to improve experimental design to make sure we got rid of such systematic bias. What we found is that each instrument sees a different picture, which is really hard for clinical purposes because you can get different outcomes in different places. The fact that MS is so variable is not quite appreciated.

Q: So where should people turn to for advice when making their buying decisions?

A: I always do a demonstration with the companies that I am interested in. I give a company one half of the sample to run and analyze and then have them analyze the other half for us in real time. We don't just get the data from them, but we want to see how easy it is to get the data. I then ask the companies for the names of their users and I go and talk to these users, which is very important. I have had quite a few people call me and I have given them forthright advice.

Q: Quality control is probably very crucial in mass spectrometry experiments, right?

A: Quality control (QC) is really important in every lab. There was a period of MS development that I refer to as the "cowboy period," when, God forbid, you repeated anything. Today when we run samples, every fourth sample is a QC sample. Anyone who is a real analyst, particularly in the small-molecule field, is already doing this. People run QC samples and standards; they keep records; they have procedures for determining whether or not to accept an assay. All this has to be translated to the proteomics field if MS has to be used as a more definitive instrument. We spent a lot of money on our laboratory information management system (LIMS) and now everything is monitored by LIMS. When an assay goes wrong, we can track back and find what could have gone wrong. People who use LIMS regularly have found it to make the reproducibility much more effective and sustainable.

Q: Is there anything you do on a more routine basis to run and maintain your instruments?

A: We have service contracts on instruments and we have people who are very well trained. We have made it more efficient and less expensive by doing some of the maintenance ourselves. The companies sometimes advise us remotely and they come in only when we have a persistent problem. We have just about halved our total service cost doing things ourselves.

Q: What are some of the biggest challenges you face today?

A: With MS, we are able to see only part of the proteome, so everyone is trying to get to high resolution, mass accuracy and speed. The outcome is not an instrument problem but an informatics issue. We are generating about 2 terabytes of data a month, on one instrument. At that level we are unable to move the data around on the computer network, and we are now in consultation with the IT group at UAB to try and transfer the data out into the cloud. I am also trying to get some funding to rewrite some of the classic programs that are used in MS into a cloud format so that the data analysis in the future will be done in the cloud. This requires some serious rewriting and then the data in the cloud can be made more secure than in your building. The other problem is we don't want to move the data around more than once. We have been doing cost analysis on what it would take to store the data on computers and that model simply won't work. We are in the same position as those people doing deep sequencing, who are probably dealing with data an order of magnitude bigger than ours. We are now talking about "deep" proteomics and "deep" lipidomics, and they all come with these huge data sets and there is going to be a huge problem with data management.