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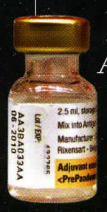

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NEW DIMENSION OF CHROMATOGRAPHY: FASTER, MORE SENSITIVE

From infant care to sports to bioterrorism, Shimadzu Corporation's chromatography instruments are enabling scientists to meet the challenges faced in an increasingly complex world.

Counterterrorism experts, pediatricians, perfumers, and professional sports watchdogs might seem, at first glance, to share few professional concerns. But many within these fields face a common challenge: the need to analyze complex biochemical substances.

For example, pediatricians, caring for infants that cannot tolerate milk need to figure exactly which protein is to blame and find substitutes. Counterterrorist experts struggle to pinpoint the origin of biological agents, which requires a precise knowledge of the subtle features that separate one bacterial strain from another. Sports regulators must monitor the growing number of natural and synthetic doping agents used by athletes to enhance their performance. The most alluring, the most fragrant perfume will not sell unless perfumers can figure out how to root out the allergens inside, and a perfume cannot be produced if the main odor-active compounds are not identified and quantified.

Scientists recruited to aid in these wide-ranging endeavors face the twin challenge of finding separation techniques that are fast enough to be of practical use and sensitive enough to provide conclusive and reliable results. The more technological progress is made, the greater scientists' demands for even more sophisticated devices. They are often forced to do so by the increasing complexity of the subject matter. For example, the new range of designer steroids used by athletes is forcing a fundamental new approach to drug screening.

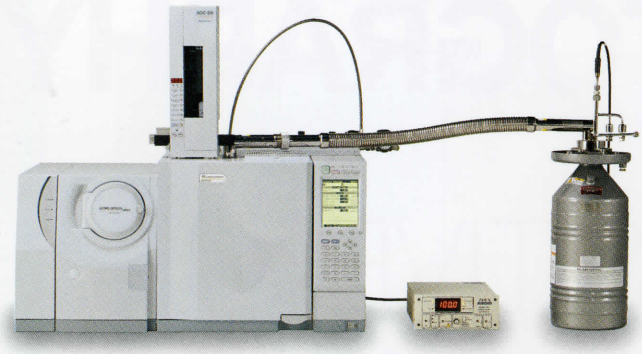
Kyoto-based Shimadzu Corporation has devoted itself to meeting this consistent demand for better performance measuring devices. It has succeeded in its mission by working directly with scientists who use them. Through such collaborations, Shimadzu Corporation engineers understand the problems and the needs from the inside, and have been able to build devices that can be finely tuned to each endeavor. The latest progress in its chromatography devices—and particularly its fast gas and liquid chromatography (GC and LC) and comprehensive two-dimensional gas, liquid or liquid-gas chromatography (GC×GC, LC×LC and LC×GC) instruments—are set to change the way we think about and approach these problems.

Collaboration born in innovation, innovation born from collaboration

Luigi Mondello
Professor
Farmaco-chimico Department
School of Pharmacy
University of Messina

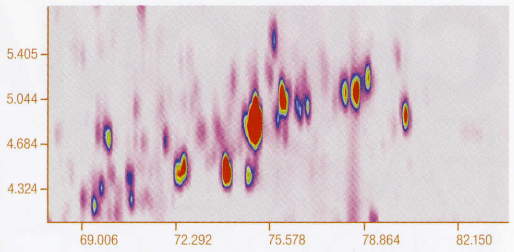
With more than 20 years of experience in the field, Luigi Mondello has become a leading light in the field of separation techniques. Aside from his hundreds of articles, in 2002, he authored a book on separation techniques, *Multidimensional Chromatography,* which has come to define the field, and another entitled *Comprehensive Chromatography Combined with Mass Spectrometry,* which will be printed in 2011. Thanks

GCxGC/qMS analysis with High-Speed Performance



GC×GC-MS System

GCxGC/qMS analysis of a fragrance extract



x-axis: retention times for first separation (in minutes)
y-axis: retention times for second separation (in seconds)

Courtesy of Prof. Luigi Mondello, University of Messina

to his rich publication record, he received the "HTC-Award for the most outstanding and innovative work in the field of hyphenated chromatographic techniques" in 2006 and the "Silver Jubilee Medal for his Considerable Contribution to the Development of Separation Sciences" given by The Chromatographic Society in 2008.

Funding agencies have recognized the importance of his work, and he now has a 1000-square-meter, multi-million Euro laboratory, fully stocked with devices that are almost exclusively from Shimadzu Corporation. "Without Shimadzu I could not have achieved all of this," says Mondello.

Mondello applies the instruments to the analysis of samples ranging from plant extracts, food products, petrochemicals, pharmaceutical and cosmetic products to various environmental substances. "In particular, the development and introduction of multidimensional instrumentation, as well as innovative software, has contributed greatly toward revealing the unsuspected complexity of many real-world samples," he says.

For example, Mondello works with the petrol industry to separate fuels. Kerosene has frustratingly long analysis times. With the new chromatography devices, Mondello has reduced processes that took two hours to two or three minutes. He has also worked with some of the world's largest perfume designers to cut through the "forest of compounds" in their newest scents to find the allergens. Under the auspices of the North Atlantic Treaty Organization, his team created a 30-minute GC×GC-MS test to identify the bacteria present in a given sample by focusing on their unique fatty lipid profiles.

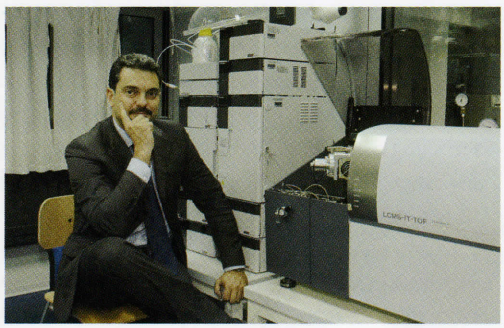
Mondello's team has also been analyzing donkey milk, which holds global potential as a substitute for bovine milk. In some 2% of children, bovine milk induces vomiting or allergic reactions. Donkey milk is much more similar in its protein and fat content to human milk, and Mondello's team provided the most detailed account yet of the triacylglycerols in it using LC×LC-MS. Based on these studies, clinical trials are now ongoing on the use of donkey milk as a substitute.

Mondello works closely with Shimadzu to develop these tools. He now focuses on ultra-fast gas chromatography that allows users to perform analyses in greatly reduced times without losing information. Fast gas chromatography units are often linked with mass spectrometers for further analysis. "This translates into the possibility of getting real time results. This is very useful when a high number of samples needs to be analyzed or immediate answers are needed," says Mondello.

Mondello and Shimadzu are also upgrading comprehensive two-dimensional and multidimensional gas chromatography. When a sample has hundreds, or even a thousand of components, conventional techniques do not have enough separation power. The two-dimensional devices automatically force the samples through two stage separation by linking two columns, each selecting for a different characteristic. The end result is another order of specificity.

Mondello says he started working with Shimadzu because of the company's willingness to completely rethink technology. "In the last ten years Shimadzu has developed entirely new instrumentations. They did not just remake old instruments," he says, adding, "I also like the Japanese way of solving problems–slowly but with excellent results."

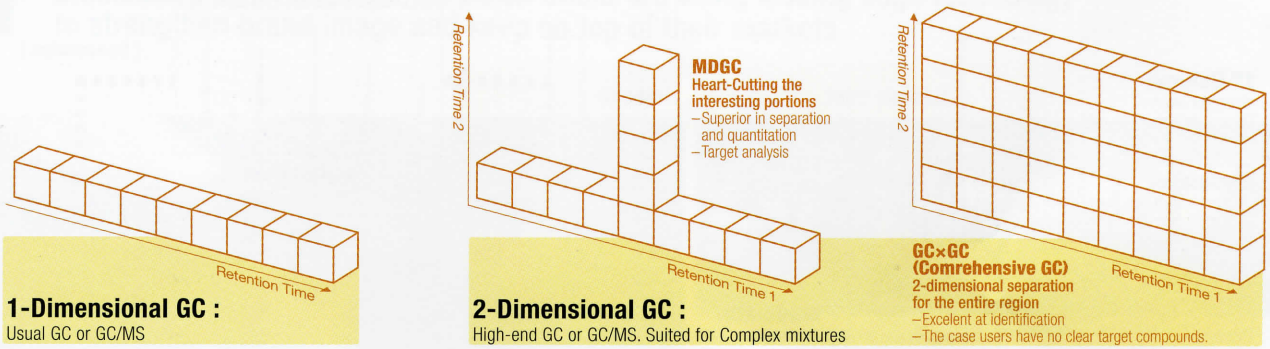
The working relationship developed into formal collaborations. Shimadzu provided instruments. Mondello's team developed software, modified the hardware, and sent back prototypes. Shimadzu then used those to make models for the market. "We tested, advised and validated these powerful devices for speed and selectivity. It has been a very nice and



Luigi Mondello with the Shimadzu LCMS-IT-TOF mass spectrometer at the University of Messina

GCxGC/qMS analysis with High-Speed Performance

Classification of Gas Chromatography



fruitful collaboration," says Mondello.

Thanks in large part to Shimadzu, both fast GC techniques have become routine over the past five to ten years. "When I first started my collaboration with Shimadzu, the use of fast GC and GC-MS was barely employed due to the lack of commercial instrumentation. Now, fast methods are routinely employed in many industrial and academic fields," says Mondello.

The same can be said for multidimensional chromatography methods. In fact, comprehensive two-dimensional gas, liquid and liquid-gas chromatography hardware and software have been developed and are now exploited by a great number of analysts across the world. GC×GC methods are making greater inroads in the analysis of fatty acid methyl esters in food and biological samples, of pesticides and petrochemicals, as well as flavours and fragrances, while LC×LC, linked with mass spectroscopy, is increasingly useful for a range of tests related to health, biology and nutrition, including proteomics, lipidomics, and food antioxidant analysis. LC×GC methods have a demonstrated effectiveness for food contaminant analysis (e.g., mineral oil in vegetable oils). "In short, analytical horizons have been extended," says Mondello.

Mondello is not one to stay complacent. He plans to continue developing instrumentation for fast GC, with attention focused on the injection system, and to develop more simple and effective modulators for comprehensive chromatography. He also plans to engage in the development of software for multidimensional chromatography and in MS spectra libraries, which will make compound identification a simpler and more reliable task. "Our main common goal, namely the evolution of chromatography-mass spectrometry technology, will be achieved within the context of our intense collaboration with Shimadzu," says Mondello.

The upshot will be far reaching. "Ten years from now, the comprehensive chromatography methods we are working with, such as LC×LC, GC×GC and LC×GC, will have a revolutionary effect on the chromatography community. If we succeed in making these powerful technologies more accessible, both in hardware and software terms, then the impact will be a great one," says Mondello.

Seeing the whole field

J. Thomas Brenna
Professor
Division of Nutritional Sciences
Cornell University

In a sporting world in which athletes are using ever more sophisticated illegal drugs to enhance their capabilities, how can a regulator keep up? The answer is by coming up with better screening techniques. Cornell University's J. Thomas Brenna has set himself on that task.

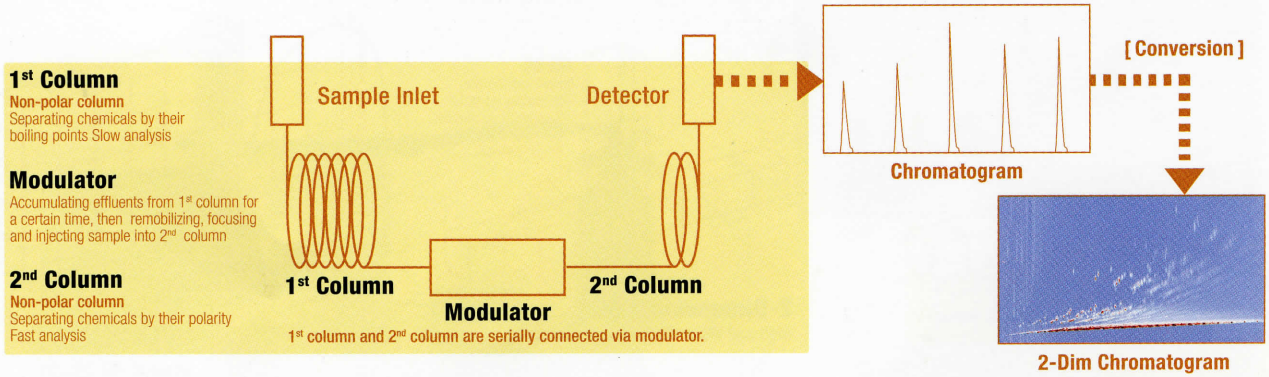
Brenna and his research group have been funded for two decades by the US National Institutes of Health and private sources for development of molecular, elemental, and isotopic mass spectrometry. His group has also been at the forefront of human polyunsaturated fatty acid nutrition and related lipid metabolism research. With that background, he and his colleagues are developing advanced techniques to help anti-doping laboratories detect use of illegal performance-enhancing substances.

Scientists have made major strides in distinguishing natural occurring testosterone from pharmaceutically derived versions. But the tests are pushed to the limits. Official doping control tests screen some 300 drugs and metabolites for each sample, and the number of necessary determinations is reaching impractically large numbers with the increasing number of samples that need to be tested and the increasing number of potential illicit drugs.

The major concern now for Brenna—and for sports regulators as well—is the threat of designer steroids which do not even have a naturally occurring counterpart. These are notoriously hard to find. Until now, the use of designer drugs was only discovered when a whistleblower turned in a syringe with the substance in it. Otherwise testers wouldn't know where to start to look.

GCxGC/qMS analysis with High-Speed Performance

Two different types of columns, modulator and special software for analysis are required for GCxGC system.



"The problem is that in a typical test, we develop a protocol, validate the protocol, and then add the test to a list. But you can't do that with a molecule you've never seen before. How do you develop tests to screen for molecules we don't suspect?" he asks.

Brenna's answer is a comprehensive screen that will match the test sample against all relevant, normally present steroids. "Imagine that steroids and other metabolites of an athlete's urine are dispersed by chromatography and by mass to make a multidimensional map with many peaks. They represent all the normally present urinary steroids and other metabolites. If you know what is supposed to be there, you can look for what's not supposed to be there. That is, we intend to establish not only where peaks are supposed to appear but also where they are not supposed to appear," says Brenna. "Then we can identify steroids or other drugs that have not been seen previously. That is what we're driving for."

But to achieve that kind of comprehensiveness and specificity, Brenna needs a powerful chromatographic device. He found it in Shimadzu's GCMS-QP2010 Plus and its GC×GC capabilities.

The device gives Brenna the separation space he needs. "Normal separation techniques work for a limited number of chemicals. Beyond a hundred or so, there is not enough space between peaks to resolve them all, let alone leave blank baseline where unusual compounds might appear. More real estate is needed along those baselines," he says.

When running a sample in Shimadzu's GC×GC technology, while the first column is running its normal 30 minute separation process, the secondary column continuously collects five-second to ten-second slices from the first and separates them again using a different chemistry. The result is much better separation even between substances that looked nearly the same from one vantage point. "Instead of two-dimensional profiles, the voluminous data appear like three-dimensional mountains," says Brenna.

The key to the speed is Shimadzu's patented quadrupole filtering device. "Their quadrupole can scan rapidly enough to keep up with the 200-millisecond-wide peaks as they emerge," he says.

The fast quadrupole provides several definitive advantages over competing technologies, such as time-of-flight (TOF) mass spectrometry. Brenna says Shimadzu's GC×GC in principle has the possibility to achieve similar sensitivity to TOF mass spectrometers at half the cost.

More importantly for scientists, the GCMS-QP2010 Plus has the capability for chemical ionization (CI), which is not available in TOFs. "We have already found that chemical ionization with specialized gases can produce sensitivity competitive with conventional electron impact ionization, but yielding far more structural specificity," says Brenna.

Brenna anxiously awaits the imminent arrival of the upgraded version, the GCMS-QP 2010 Ultra. "We'll anticipate even better data," he says.

That won't be welcome news for those designing new illegal drugs. Brenna is already analyzing crude extracts of urine to quantify steroids, taking advantage of the very high separation space available with GC×GC-qMS. "We expect to be able to see many more steroids than previously possible and perhaps unexpected steroids," he says.

It won't be easy to find a way around this new test. Designing something with anabolic activity that would be undetectable will be difficult. "In principle, a rational strategy to defeat a doping test is to design a novel illegal substance with very similar separation properties to a normal, endogenous compound, which would thus be hidden from view. We hope to make that task much more difficult using the vastly greater separation space of GC×GC-qMS," says Brenna. He adds: "Shimadzu has given us a weapon that I didn't expect to have."

Despite this progress, chromatography has not reached its end. Mondello poses himself the question: how much more room is there for development both in the LC and GC hardware and software field? Have we reached the maximum level of separation power, selectivity, sensitivity and speed? "I have an answer to this, which is simply no. There's still plenty of room for development, before the 'perfect' GC or LC method is developed," he says. But with Shimadzu's ongoing efforts, that goal will always be coming closer.