Easy, reliable

and completely integrated microfluidic nanospray LC/MS

Agilent 1200 Series HPLC-Chip/MS System

Our measure is your success.

products | applications | software | services

Agilent Technologies

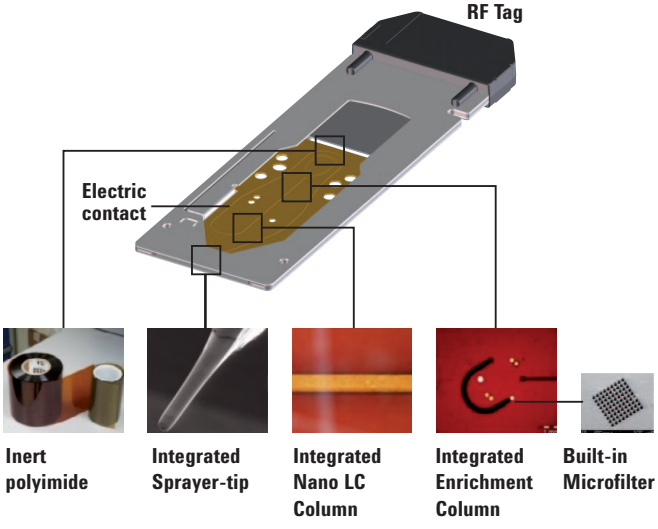
Agilent 1200 Series HPLC-Chip II and 6000 Series MS

Clearly better together

The Agilent 1200 Series HPLC-Chip/MS system is based on revolutionary microfluidic chip technology specifically designed for nanospray LC/MS. The system includes 1200 Series Capillary and Nanoflow Pumps, Micro-well Plate Sampler with Thermostat, Chip Cube MS interface, and any Agilent 6000 Series Mass Spectrometer. System control is through either Agilent ChemStation or MassHunter software. The second generation HPLC-Chip technology incorporates a carbon ion implanted filter, improving surface characteristics dramatically for optimal contact and sealing, as well as reducing friction between rotor and polyimide chip. These improvements double chip lifetime, lower the cost per analysis as well as improve chip-to-chip and run-to-run reproducibility. With Agilent's HPLC-Chip II, the Agilent HPLC-Chip/MS system takes you to new levels of nanospray MS reliability, robustness, sensitivity and ease of use, and empowers you to enter new application frontiers:

- **Biomarker discovery and validation**
- **Intact monoclonal antibody characterization**
- **Small molecule analysis, such as DMPK**
- **Phosphopeptide analysis in post-translational modification (PTM) studies**

HPLC-Chips are available for a wide range of application needs. Second-generation technology with new carbon ion implanted filter gives improved surface characteristics for optimal contact and sealing, as well as reduced friction between rotor and polyimide chip.



A complete portfolio of LC solutions

Agilent offers the broadest portfolio of liquid chromatography solutions. Choose from systems for analytical HPLC applications or move up to high performance solutions for capillary, nanoflow or preparative LC, and for HPLC-Chip/MS or UHPLC.

To learn more, visit
www.agilent.com/chem/lc

Phosphochip for Agilent 1200 Series HPLC-Chip/MS

One-step phosphopeptide analysis

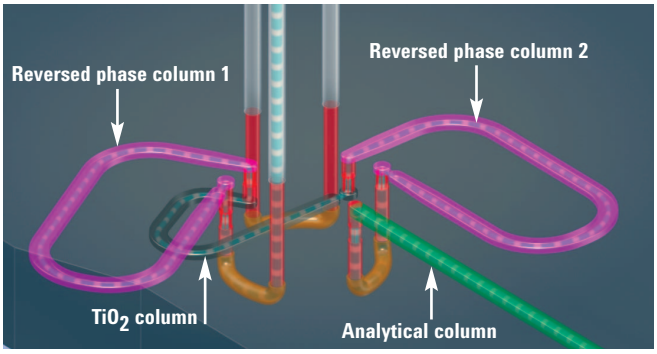
Moving beyond protein identification, more and more researchers are focusing on the protein phosphorylation mechanism, an important post-translational modifications (PTMs) process, to understand signal transduction in the cell. To investigate the phosphoproteome in more detail, phosphorylated proteins and peptides of interest need to be enriched prior to LC/MS/MS analysis due to the low abundance of phosphorylated proteins in complex mixtures.

Phosphochip – simplifies your workflow

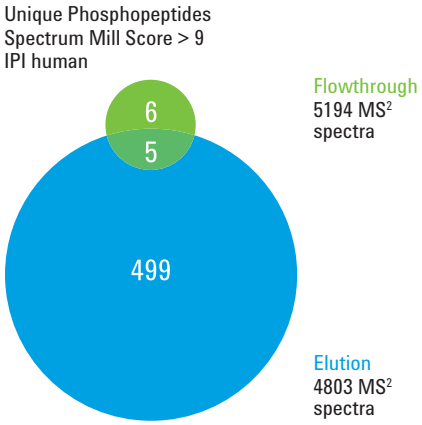
- Multilayer microfluidic HPLC-Chip features a sandwiched RP-TiO_2-RP trapping column (shown pink-black-pink in the figure) for phosphopeptide enrichment
- Dual modes of analysis of both phosphorylated and non-phosphorylated peptides from complex protein digest (see workflow)

Large scale identification of phosphopeptides from complex samples

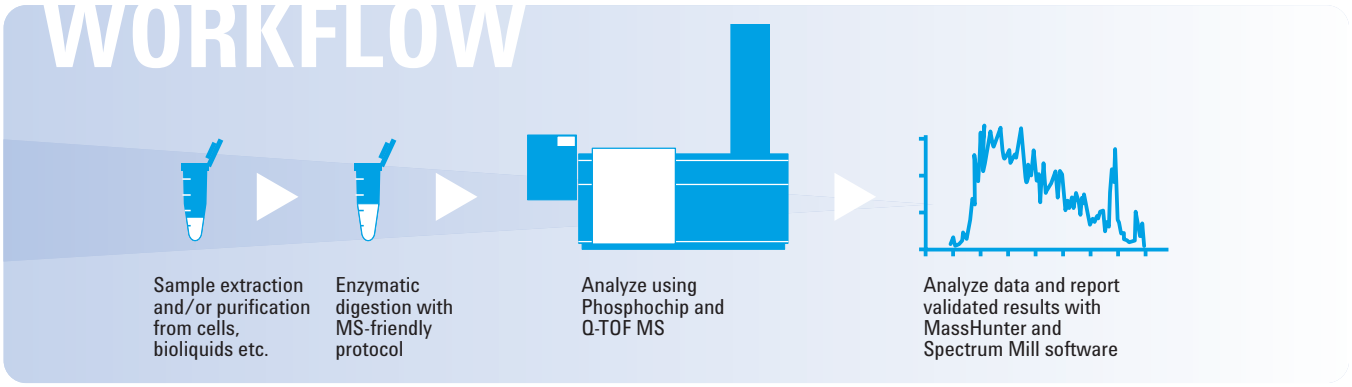
- Phosphochip incorporates classic phosphopeptide enrichment approach in an efficient and easy-to-use HPLC-Chip system
- Large number of phosphopeptides can be identified from complex digest of human cells



The HPLC-Chip seamlessly integrates the sample enrichment and analytical columns with the intricate connections and spray tip directly on the polymer chip.



Phosphochip facilitates identification of large numbers of phosphopeptides from complex human cell digests (data courtesy of Prof. Albert Heck, Utrecht University, Netherlands).



HPLC-Chip/MS for protein discovery to targeted quantification

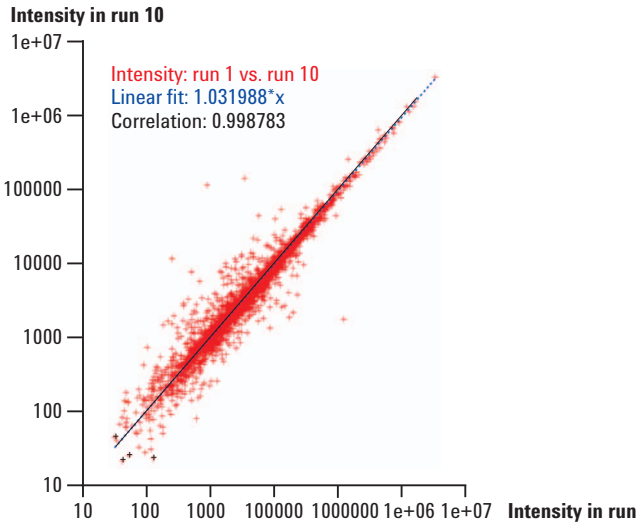
Protein biomarker discovery and validation

Protein biomarker discovery

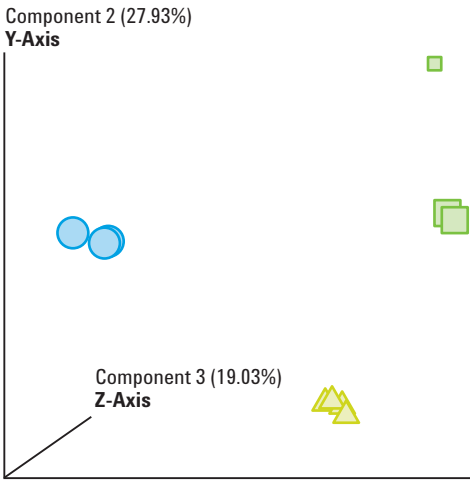
Biomarker discovery and validation is the first step in the new paradigm of drug development involving biotechnology. Differential protein expression analysis is employed in putative protein biomarker determination in clinical research or pharmaceutical development. Featuring HPLC-Chip/MS, Agilent offers a label-free identification and relative quantification workflow for rapid screening of potential biomarker and subsequent targeted validation of biomarker candidacy.

Reproducibility is key for biomarker discovery

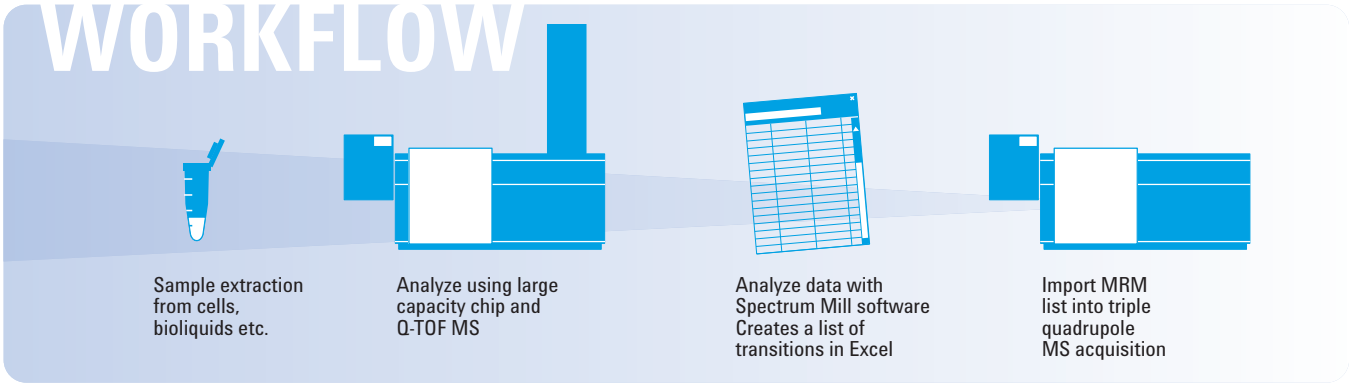
- **MS abundance reproducibility** is necessary for ensuring that analytical variability remains well within the biological variability. The integrated spray design on the HPLC-Chip facilitates a stable nanospray and in combination with the 6500 Series Q-TOF design provides for excellent abundance reproducibility.
- **Retention time (RT) reproducibility** is also critical to ensure that molecular features align from replicate LC/MS runs. The HPLC-Chip delivers minimal retention time variation of the order of a few seconds over an hour long gradient.
- **More accurate statistical analysis**, such as principle component analysis (PCA), can be achieved from data obtained from complex sample analysis – a direct benefit from the combined abundance and RT reproducibilities of HPLC-Chip/MS.



Scatter plot of ion intensities from 2 runs of 5000 glycopeptides. (data courtesy of Prof. Rudi Aebersold, Institute of Molecular Systems Biology, Switzerland)



PCA analysis of complex digest samples reveal differentiated features among sample sets

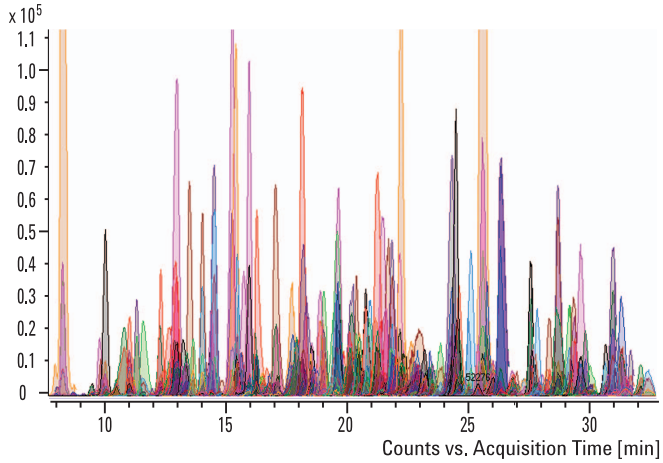


Protein biomarker validation

Confirmation of putative protein biomarkers in complex biological samples requires an instrumental method that is fast, highly selective and sensitive. Peptide quantification using multiple reaction monitoring (MRM) has been established as an important methodology for biomarker validation.

Easy transition from discovery to validation

- The plug and play aspect of the Agilent HPLC-Chip enables the use of the same chip for discovery as well as validation thus ensuring retention integrity being retained
- The shared axial acceleration collision cell between Agilent quadrupole time-of-flight and Agilent triple quadrupole mass spectrometers enables the data from the discovery phase to be used to set up the time windows for the dynamic MRM process on the triple quadrupole system
- The high quality retention time reproducibility allows the use of very narrow windows with the dynamic MRM feature.



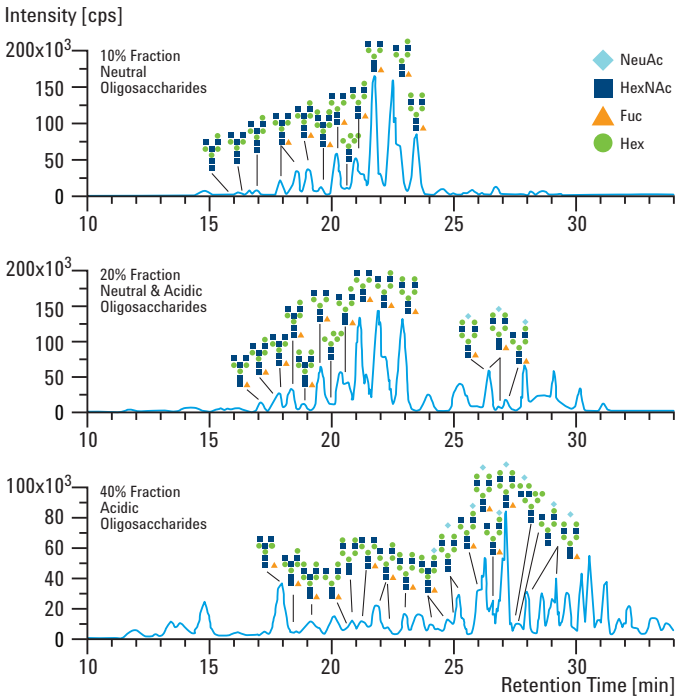
More than 2000 transitions can be acquired in a single dynamic MRM experiment using HPLC-Chip coupled to a triple quadrupole mass spectrometer.

Glycans as biomarkers

Confirmation of putative glycan biomarkers in complex biological samples can also be analyzed. PGC-Chip with graphitized carbon materials provides selectivity in oligosaccharide analysis.

Clinical glycomics

- Targeting N-linked glycans released from human serum for biomarker discovery
- Detecting oligosaccharides for a particular protein
- Reproducible, sensitive and accurate HPLC-Chip/MS analysis for acidic and neutral glycans



Reproducible, sensitive and accurate PGC-Chip/Q-TOF analysis for acidic and neutral glycans (data courtesy of Prof. Carlito Lebrilla, University of California at Davis, USA).

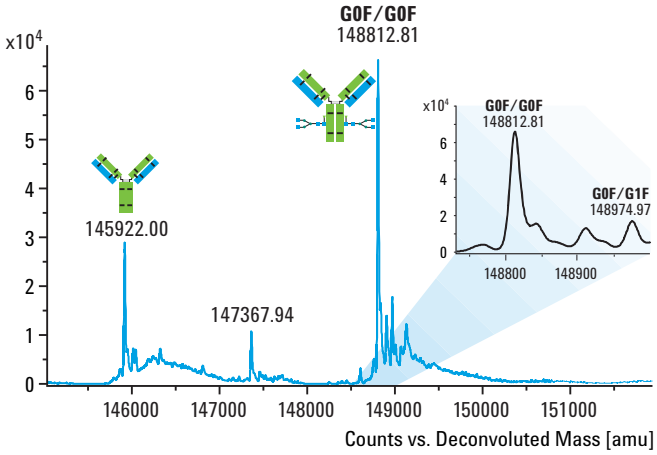
To learn more about the Agilent 1200 Series HPLC-Chip, visit www.agilent.com/chem/hplc-chip

Rapid identification of protein heterogeneity

Analysis of monoclonal antibodies

MAbs glycosylation stoichiometry

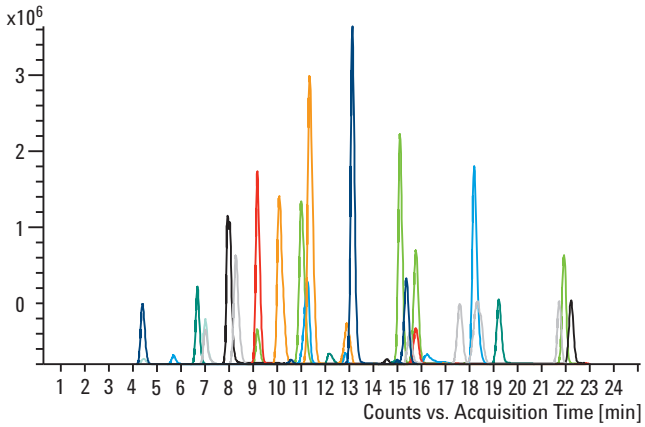
The routine analysis of biopharmaceutical proteins, as well as purified proteins used in basic research, can be performed reliably with very small amounts of sample using the HPLC-Chip/MS system. For example, recombinant monoclonal antibodies (MAbs), which have therapeutic applications, are relatively stable biomolecules, but a number of chemical modifications and degradation reactions can occur during their manufacture, formulation and storage. Accurate mass measurement of the intact antibodies and/or their subunits can be useful for rapid verification of their integrity. The high sensitivity and sub-10 ppm mass accuracy of the HPLC-Chip/Q-TOF or TOF MS system can enable rapid identification of protein heterogenity and confirmation of glycosylation states, while requiring almost 1000 times less sample than conventional HPLC methods.



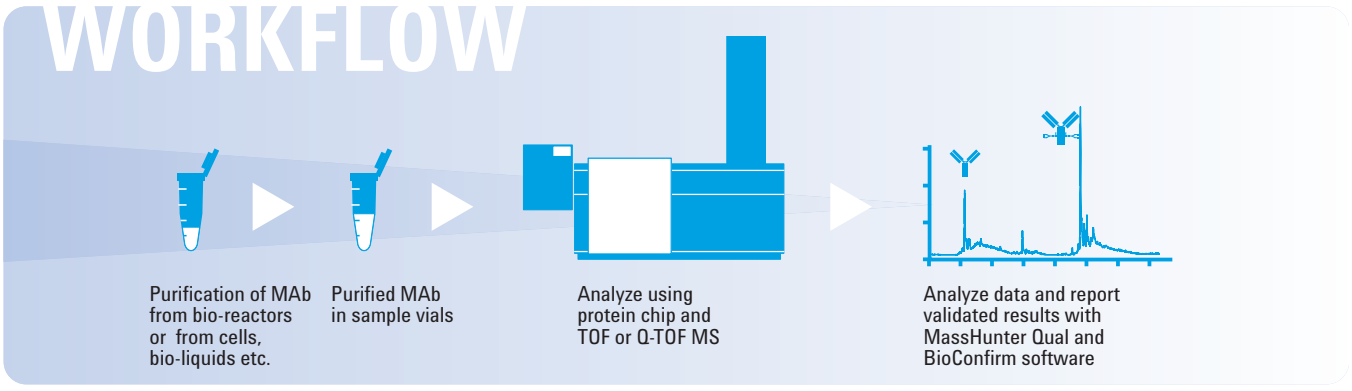
Deconvoluted mass spectrum of intact MAb reveals glycosylation stoichiometry.

MAb sequence confirmation

Peptide mapping using LC/MS is an established analytical tool for the confirmation of MAb amino acid sequence in quality control. HPLC-Chip with quadrupole time-of-flight MS can be used to analyze MAb tryptic digest followed by data analysis using BioConfirm tool within the MassHunter Qualitative Analysis software. This combination of easy tools can yield high sequence coverage with high mass accuracy, usually below 5 ppm.



Extracted ion chromatogram (EIC) of some peptides from MAb light chains and heavy chains.



Low level quantification in complex biological matrices

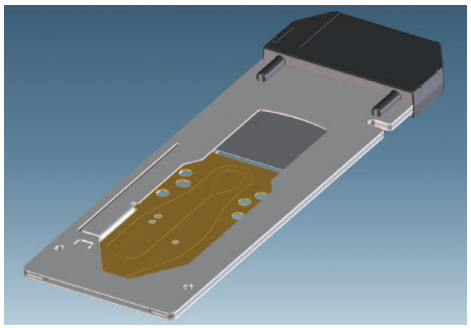
Analysis of small molecules

Enhanced sensitivity through UHC-Chip

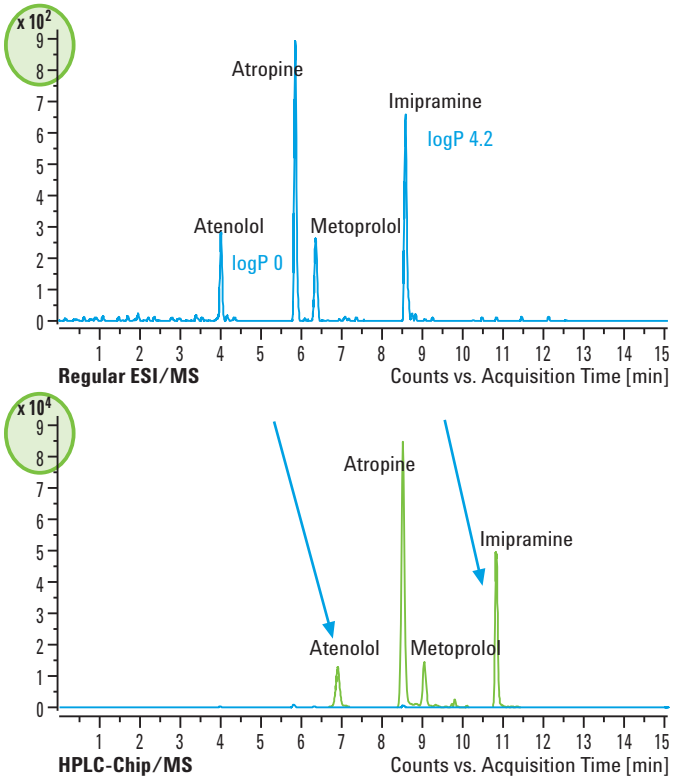
The Agilent ultrahigh capacity HPLC-Chip (UHC-Chip) is ideal for drug metabolism and pharmacokinetic (DMPK) studies where the volume of blood diminishes. The reduction of rodent size and number is accompanied by a cutback in the amount of the active pharmaceutical entity per PK study. This ultimately leads to substantial cost-savings in the DMPK laboratory. Small animals can only donate a limited sample volume in particular when serial sampling studies are performed to increase PK data quality. The combination of the UHC-Chip and triple quadrupole MS provides enhanced sensitivity and robustness of low level quantification of compounds of interest from complex biological matrices, specifically from less than 10 µL of blood sample.

UHC-Chip with triple quadrupole MS for DMPK

- Delivers 100x increase in sensitivity comparing to the conventional electrospray at higher flow rate
- Equally effective in analyzing compounds with a wide range of hydrophilicity
- Compatible with the emerging dried blood spot sample preparation method

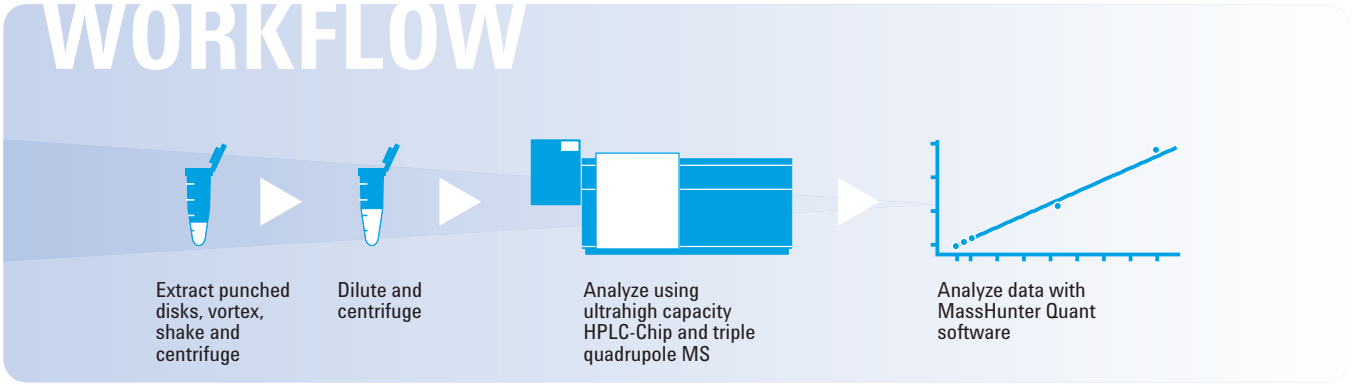


Agilent UHC-Chip with a 500 nL enrichment column.



Direct comparison of regular ESI and HPLC-Chip shows a significant boost in sensitivity when a HPLC-Chip/MS system is used.

WORKFLOW



Custom HPLC-Chip enables new research in glycan expression

Case study: Boston University

"The HPLC-Chip was an enabling technology for us."

Progress in biomedical research often takes a quantum leap when the right instrumentation becomes available. Glycan expression is one of those examples. Structurally diverse glycans are found on mammalian cell surfaces and in extracellular matrices. They mediate cell-cell recognition and cell-cell and cell-matrix interactions, but the exact details are largely a mystery. An innovative Agilent HPLC-Chip has now solved many of the difficulties that were inherent with LC/MS characterization of these compounds.

Professor Joseph Zaia and his team at the Mass Spectrometry Resource within the Boston University School of Medicine develop LC/MS methods to study the range of glycans that are expressed under various cell conditions. While nanoflow LC/MS with negative ion electrospray is the method of choice for characterization and quantification of these diverse carbohydrates, researchers must overcome a number of challenges. We spoke with Professor Zaia about the impact the HPLC-Chip has made in his research.

Rapid setup and stable performance

Before the HPLC-Chip, the lab's major problem resided in the interface between the LC and the MS. Achieving a stable spray in negative ion mode at nanoflow rates was difficult, so it took a long time just to set up an experiment. That all changed the first time they used the HPLC-Chip – a small device with microfluidic channels, HPLC column packings, an integral nanoelectrospray emitter, and electrical contacts for electrospray. Zaia commented, "We quickly realized that the HPLC-Chip was engineered to solve this problem, and that it was no longer going to be a struggle to acquire the data. The HPLC-Chip was an enabling technology for us."

Based on this initial success, Zaia's group purchased an Agilent HPLC-Chip/MS system that included an Agilent 6520 Accurate-Mass Q-TOF LC/MS. At about the same time, they applied for and received a university grant from the Agilent Foundation, which began a collaborative effort that has introduced new capabilities to the scientific community.

Makeup flow enables overnight runs

Zaia explained that his team uses hydrophilic interaction liquid chromatography (HILIC), which requires a custom chip with a special packing material. The gradient runs from high to low organic, which ordinarily makes it difficult to get a stable spray in negative mode at the end of the run. To solve this problem, Agilent collaborators provided an experimental makeup flow (MUF) HPLC-Chip, which allows Zaia's team to add organic solvent postcolumn. They achieve a consistent spray over the entire gradient, which extends the range of glycans they can measure. And they no longer need to adjust source voltages during the run, so they can now perform overnight, unattended analyses.

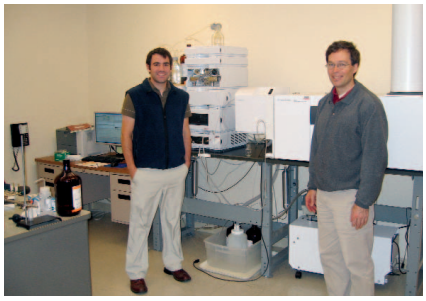
Now possible: analysis of large sample sets

Professor Zaia spoke about the large-scale studies he can now conduct to examine differences in the temporal and spatial expression of glycosaminoglycans (GAGs). For this critical research, the LC/MS system must provide consistent, reliable data for triplicate runs of dozens of samples. "The system has to be stable for days to get through the set of samples we need to handle. Over the past year and half, we've been able to use the system for these large sample sets. Previously, we would not have had the instrument stability to address these research questions."

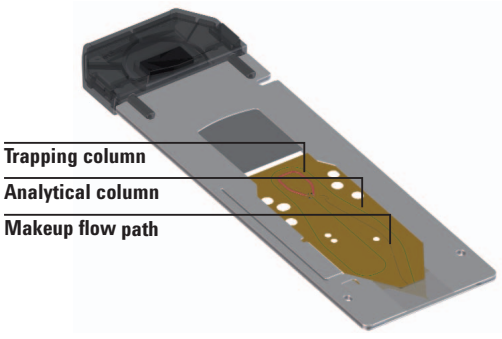
A new way to sequence fragile GAGs

The team is excited about future plans to further characterize GAGs using LC/MS/MS. GAGs are particularly challenging because many are sulfated, which makes them quite labile. Zaia plans to use the makeup flow to introduce an additive that will stabilize the sulfate group so he can get useful backbone fragmentation without loss of side groups.

He cites the continued collaboration with Agilent as a key to future success. "Agilent has been very supportive in working with us on this technology. We look forward to the move into online tandem MS to do more detailed characterization."



Graduate student Greg Staples (left) and Professor Joseph Zaia (right) use this Agilent HPLC-Chip/MS system for leading-edge research on glycoconjugates.



This HPLC-Chip incorporates a makeup flow, which helps to achieve stable electrospray when the LC gradient provides less than ideal conditions.

Powerful hair-based drug testing technology using HPLC-Chip/MS

Case study: Hong Kong University

"This hair-based test provides the answer to a range of technical challenges."

A Hong Kong University of Science and Technology (HKUST) professor has successfully developed a hair-based drug testing method using the Agilent 1200 Series HPLC-Chip/MS system with an Agilent 6410 Triple Quadrupole LC/MS. The new method is an alternative to other drug testing methods, including the urine-based method commonly used in Hong Kong. The hair-based method is 1,000 times more sensitive than the urine-based method that is often used in China and the United States. The hair-based drug testing technology using the HPLC-Chip/Triple Quadrupole system can trace a person's drug-taking history over the past 3 months or more, in terms of what drugs have been taken, when, and the actual dosage.

Prof Karl Tsim, Head of the Department of Biology at HKUST, has spent over a year developing this new technology. "This hair-based test provides the answer to a range of technical challenges associated with the urine-based drug testing technology commonly employed in Hong Kong," he said. Compared with urine-based drug testing, the new hair-based method solved some of the challenges with sample collection, storage, and reliability.

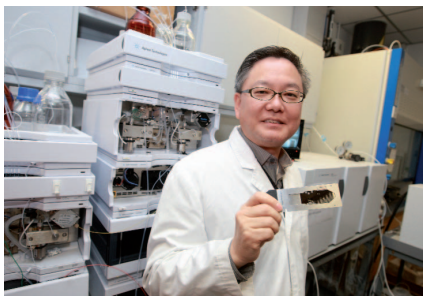
Prof Tsim said, "While our technology addresses many of the challenges of the urine-based drug test, admittedly it also has certain limitations. First, it can only detect drugs taken 7 or more days ago. Also, the time taken to yield results – one to two days – is longer than the urine-based drug test where the results are shown almost immediately. And currently the cost for our test is somewhat higher than that for a urine-based test." "Hence our technology is not intended to replace the urine-based test, but can serve as a powerful supplement to it," he added.

Currently, other hair-based drug testing technologies are being used in China and the USA. Most of these technologies are based on GC/MS, and have certain drawbacks. For instance, for GC/MS the hair samples need to be treated by following a rather complicated set of procedures. Moreover, the samples need to be of larger quantity – about 50 strands of hair. On the other hand, for the HPLC-Chip/ Triple Quadrupole technology developed by HKUST, only a simple drug extraction step is needed, and the sample quantity required is much lower – only 5 to 10 strands of hair. This is because HPLC-Chip/ Triple Quadrupole is 1,000 times more sensitive than GC/MS for the analysis of drugs like heroin, cocaine, ketamine, and methadone in hair. Prof Tsim and team are working on a manuscript to publish their work in a scientific journal.

	Urine-based testing	Hair-based testing
Sample Collection	• Need designated place by and personnel • Embarrassing process	• Easy – only 5-10 strands of hair • Do need hair
Sample Storage	• Low temperature	• Room temperature
Test Targeting Period	• Targets drugs taken within past several days • No drug-taking history preceding this period	• Trace drug-taking history from 7 days to 3 months • Possible to trace dosage, thus degree and pattern of addiction
Results Quality	• Tests sensitive to certain Western and Chinese herbal medicines, resulting in "false positive" • Easy to create "false negative" outcome, by taking water or electrolyte drinks	• Not likely to generate "false positive" or "false negative" results • May also be applicable to new types of drugs that cannot be revealed by many of the urine tests currently used

LC/MS/MS and other solutions for drug testing

Hair-based drug testing has several advantages over urine-based testing, including the ease of sample collection and the ability to gather historical data. The Agilent HPLC-Chip/MS technology, in combination with the Agilent 6400 Series Triple Quadrupole, delivers impressive sensitivity for this analysis. Agilent has for decades provided products for drug testing, and has a rich portfolio of solutions that span sample preparation to data management. If you are working with drug testing methods, learn more about Agilent solutions on our drug testing Web page.



Professor Karl Tsim showing an HPLC-Chip used for his drug testing procedures.

Agilent 1200 Series HPLC-Chip/MS

Application examples and citations

Applications

A complete list of applications using Agilent HPLC-Chip/MS systems is available on our web site at **www.agilent.com/chem/hplc-chip**

Gudihal R, Ragalopalan S, Gulge R, Gieschen A, Miller C, Tang N and Waddell K, "Primary Characterization of a Monoclonal Antibody Using Agilent HPLC-Chip Accurate-Mass LC/MS Technology," *Agilent pub. no. 5990-3445EN*

Gudihal R and Waddell K, "Peptide Mapping of a Monoclonal Antibody using a Microfluidic-based HPLC-Chip coupled to an Agilent Accurate-Mass Q-TOF LC/MS," *Agilent pub. no. 5990-4587EN*

Buckenmaier S and Bonvie A, "HPLC-Chip/Triple-Quadrupole MS for quantification of pharmaceuticals in diminishing small volumes of blood," *Agilent pub. no. 5989-9896EN*

Raijmakers R, Mohammed S and Heck AJR, "Facilitating Phosphopeptide Analysis Using the Agilent HPLC Phospho-chip," *Agilent pub. no. 5990-4098EN*

Buckenmaier S and Vollmer MD, "Using nanospray HPLC-Chip/TOF for routine high sensitivity metabolite identification," *Agilent pub. no. 5989-5938EN*

Gudihal R and Waddell K, "Glycopeptide and glycan analysis of monoclonal antibodies using a microfluidic-based HPLC-Chip coupled to an Agilent Accurate-Mass Q-TOF LC/MS," *Agilent pub. no. 5990-5190EN*

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Thibault P, "The Agilent HPLC-Chip/6210 TOF LC/MS Enables Highly Accurate Profiling of Peptide Maps for Differential Expression Studies," *Agilent pub. no. 5989-5084EN*

Citations

A complete list of papers from peer-reviewed proteomics publications using Agilent HPLC-Chip/MS systems is available on our web site at **www.agilent.com/chem/hplc-chip**

Staples GO, Naimy H, Yin H, Kileen K, Kraiczek K, Costello CE, and Zaia J, "Improved hydrophilic interaction chromatography LC/MS of heparinoids using a chip with postcolumn makeup flow," *Anal. Chem.* **2010**, 82(2):516-22

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Trusch M, Böhlick A, Hildebrand D, Lichtner B, Bertsch A, Kohlbacher O, Bachmann S, and Schlüter H, Application of displacement chromatography for the analysis of a lipid raft proteome. *J Chromatogr B Analyt Technol Biomed Life Sci.* **2010**, 878: 309-314.

Haruta M, Burch HL, Nelson RB, Barrett-Wilt G, Kline KG, Mohsin, SB, Young JC, Otegui MS, and Sussman MR, Molecular Characterization of Mutant Arabidopsis plants with reduced plasma membrane proton pump activity. *J Biol Chem.* **2010**, Mar 26 (online publication ahead of print).

Agilent 1200 Series HPLC-Chip/MS

Customer testimonials

“The Phosphochip is the ultimate ease-of-use tool for phosphopeptide analysis as it has a fully integrated microfluidic design that enables routine phosphopeptide analysis, without the hassles of clogging and plumbing in conventional nanoflow LC”
Professor Albert J.R. Heck, Utrecht University, Netherlands

“For long time veterans of nanospray and proteomics, the Agilent HPLC-Chip is like Christmas morning. Forget the fused silica, the flame-pulled tips and pressure cell packing. Just slide in a spray chip and you’re good to go!”
David K. Crockett, ARUP Institute for Clinical and Experimental Pathology, USA

“Using HPLC-Chip, we have more than doubled our productivity, analyzing four to six samplers per hour instead of the one or two that we could do with classical nanoflow LC, and all at increased sensitivity.”
Dr. David Stapleton, Bio21 Molecular Science and Biotechnology Institute, Australia

“The HPLC-Chip represents a significant advance in the separation tools available to proteomics researchers."
Professor Rudolf Aebersold, Institut für Molekulare Systembiologie, Switzerland

“The reproducibility and sensitivity of the HPLC-Chip/nanoflow LC coupled to either an ion trap or time-of-flight mass spectrometer provides a powerful protein identification and expression platform to any proteomics and drug discovery programs."
Professor Pierre Thibault, Université de Montréal, Canada

"It is easy to perform nanoflow-LC ES-MS analysis using the Agilent HPLC-Chip system. Not only does it simplify the process but also produces more reliable data."
Prof. Ming-Ren Fuh, Soochow University, Taiwan “

The primary problem for LC/MS glycomics has been the difficulty in achieving stable and sensitive nanoscale electrospray over the long time periods required for analysis of large sample sets. The Agilent HPLC-Chip/MS system solves this problem by virtue of an engineered device that contains the trapping cartridge, nano-column, microfluidic connections, and electrospray emitter."
Prof. Joseph Zaia, Boston University Center for Biomedical Mass Spectrometry, USA

"Scientists should spend their time in the lab doing meaningful biological experiments, not searching for leaks and dead volumes in their nanoflow-LC system. The Agilent HPLC-Chip is the solution, making nanoflow-LC for high sensitive protein and PTM identification easy, reliable, stable, and reproducible, far exceeding the traditional setup."
Dr. Manfred Raida, Experimental Therapeutic Center, Singapore

"I love the Agilent Chip Cube set up! Before we moved to the Chip Cube, nanospray was such a pain and it could only be managed by experienced users. Now, we can train someone to run their own samples in a few hours. ."
Prof. Brian Bothner, Montana State University, USA

"The Agilent Chip Cube is wonderful for our facility, since we train graduate students, postdocs and technicians to run their own samples and to understand the analysis process in more detail than would be possible in a service mode."
Prof. Edward Dratz, Montana State University, USA

“The reproducibility and reliability of the Agilent HPLC Chip system combined with the power of repetitive exclusion runs on the 6500 Q-Tof has allowed me to dig deeper into proteomes than I ever thought was possible.”
Dr. Nicolas L Taylor, University of Western Australia, Australia

"The introduction of the HPLC chip technology represents a quantum leap in the development of nano-LC. The key benefit to our laboratory has been the significant increase in productivity since people with no experience in nano liquid phase separations readily can perform nano-ESI-MS analysis on a routine basis using the Agilent chip system."
Dr. Tasso Miliotis, AstraZeneca, Sweden

To learn more about the Agilent 1200 Series HPLC-Chip, visit www.agilent.com/chem/hplc-chip

HPLC-Chip portfolio

Application		Order Number	Description
Calibration and Maintenance	Calib-Chip (II)	G4240-61010	MS calibration and diagnostic chip
	FIA-Chip (II)	G4240-61015	Dedicated infusion and flow injection chip
Proteomics	ProtID-Chip-43 (II)	G4240-62005	43 mm 300 Å C18 chip with 40 nL trap column
	ProtID-Chip-150 (II)	G4240-62006	150 mm 300 Å C18 chip with 40 nL trap column
	Large Capacity Chip (II)	G4240-62010	150 mm 300 Å C18 chip with 160 nL trap column
	Phosphochip (II)	G4240-62020	150 mm 300 Å C18 chip with RP-TiO_2-RP trap column
Small Molecule Analysis	SmMol-Chip-43 (II)	G4240-65005	43 mm 80 Å C18 chip with 40 nL trap column
	UHC-Chip (II)	G4240-62010	150 mm 80 Å C18 chip with 500 nL trap column
Other Application	PGC-Chip (II)	G4240-64010	43 mm porous graphitized carbon chip with 40nL trap column
Custom Chips	Dir-Inj-Chip (II)	G4240-63001 SPQ 100	150 mm 300 Å C18 chip with 16 nL void
	Protein Chip (II)	G4240-63001 SPQ 105	43 mm 300 Å C8 chip with 40 nL trap column
	Custom Chip	Special SPQ Number	Design your own chip and ask for a special quote

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