Agilent 1100 Series HPLC-Chip/MS system

The easy-to-use HPLC-Chip for reliable high sensitivity nanospray LC/MS

The Agilent 1100 Series HPLC-Chip/MS system is a new revolutionary microfluidic chip-based technology specifically designed for nanospray LC/MS. Based on Agilent's new groundbreaking HPLC-Chip and HPLC-Chip Cube MS interface, the Agilent HPLC-Chip/MS system takes you to a new level of nanospray MS sensitivity, robustness, reliability, and ease of use.

Nanospray LC/MS is well established as state-of-the-art technology because of its high sensitivity and low sample consumption. It is most often used for applications with limited sample amounts or when there is a need for analysis of trace level components in complex mixtures.

Although nanospray LC/MS provides very high sensitivity, the use of multiple small capillary tubing connections and frequent clogging or leaking at the columns and nanospray needle make it challenging to implement and maintain. The Agilent HPLC-Chip/MS system solves these problems by integrating all of these components directly onto a reusable biocompatible polymer HPLC-Chip. Solvent and sample delivery to the chip, high pressure switching of flows and automated chip loading and positioning in the MS source are accomplished by the new HPLC-Chip Cube MS interface module.

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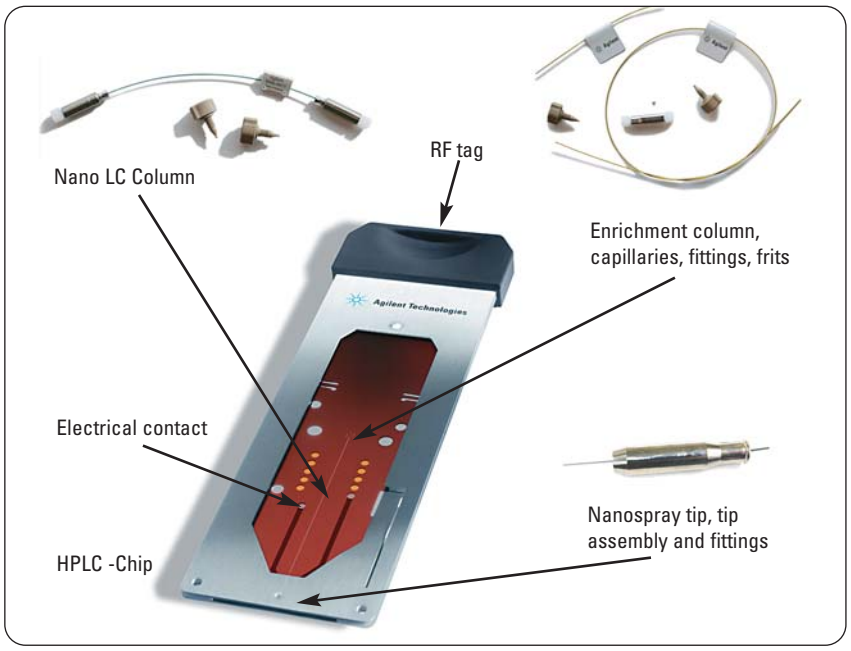
Agilent HPLC-Chip and Agilent HPLC-Chip Cube Interface

The HPLC-Chip

The reusable HPLC-Chip seamlessly integrates the sample enrichment and separation columns of a nanoflow LC system with the intricate connections and nanospray tip used in electrospray mass spectrometry directly on a biocompatible polymer chip. This eliminates the traditional fittings, valves and connections typically required in a nanospray LC/MS system. This dramatically reduces the possibility of leaks and eliminates all post-column dead volumes. Peak dispersion is virtually eliminated, resulting in narrower, better-defined peaks and greatly improved separations. For concentration dependant MS detectors, this will also increase sensitivity. Overall robustness, reliability, and ease of use are greatly improved. The complete integration of the chip and zero dead volume connections between all components ensures uncompromised chromatographic performance and delivers the full separation efficiency of the analytical column, allowing you to identify more compounds in complex samples. The chip integrates all electrical connections and features an imbedded radio frequency (RF) tag that tracks usage and operating parameters. Information on the RF tag is automatically downloaded and updated by the system software.



Integrated HPLC-Chip eliminates tedious and complex connections and delivers uncompromised chromatographic separations.

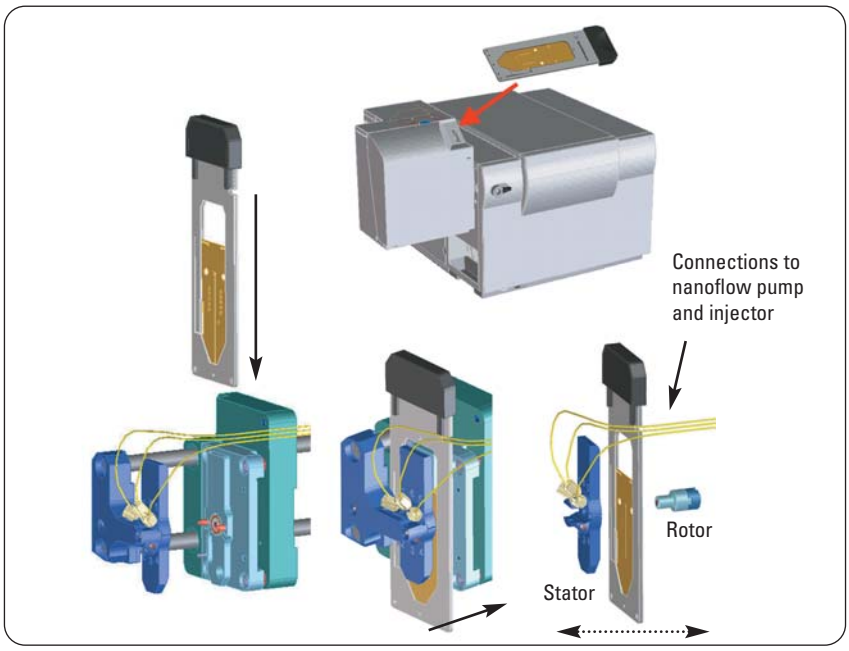
The HPLC-Chip Cube Interface

The HPLC-Chip Cube MS interface contains the loading mechanism for chip positioning, the microvalve for nano-LC hydraulic connections and flow switching, and the nanospray ion source with camera for spray visualization. The HPLC-Chip Cube automatically and precisely positions the chip spray tip orthogonal to the MS inlet to ensure maximum sensitivity and robustness, and makes the necessary electrical connections and all hydraulic connections to the chip. The entire process is fully automated and requires no tools. Since it's so easy to do, each individual researcher can have his or her own dedicated chip, reducing the risk of cross contamination.

HPLC-Chip Cube interface.

Automatic HPLC-Chip loading and hydraulic connections eliminate fittings and connection capillaries

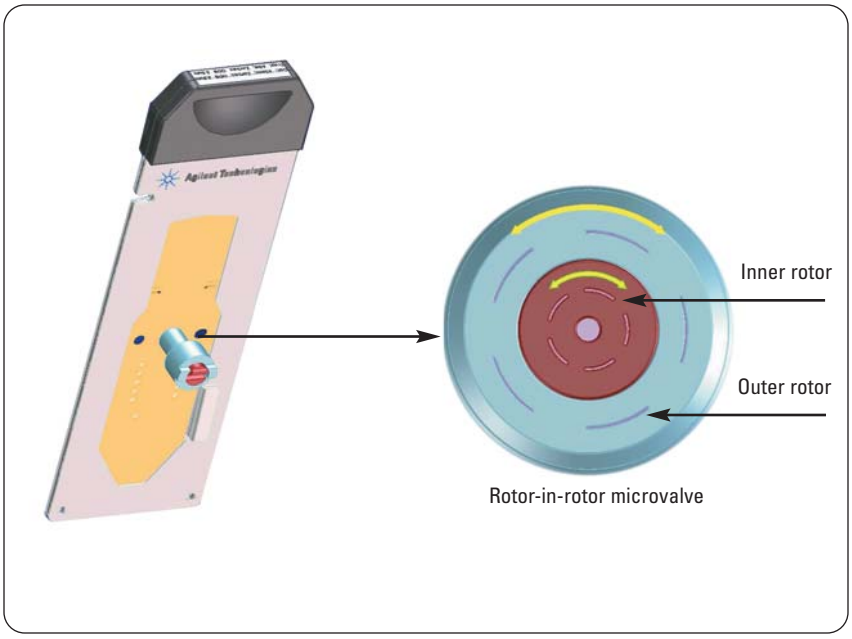
With the Agilent 1100 Series nanoflow LC, micro well-plate autosampler, and capillary sample loading pump directly connected to the HPLC-Chip Cube, the chip is automatically loaded and leak-tight fluid connections are established by sandwiching the chip between the rotor and stator of the built in multiport microvalve. The rotor and stator dock onto the chip and align the flow path from the nano-LC and loading pump to the port on the chip surface. Moving the rotor ensures quick and reliable switching between different flow paths such as sample enrichment and sample analysis paths.



The HPLC-Chip Cube automatically loads the chip, establishes leak tight connections and positions the chip orthogonal to the MS inlet simply by clicking on the operate command in the Chemstation menu.

Unique rotor-in-rotor microvalve for fluid delivery to multi-layered HPLC-Chip

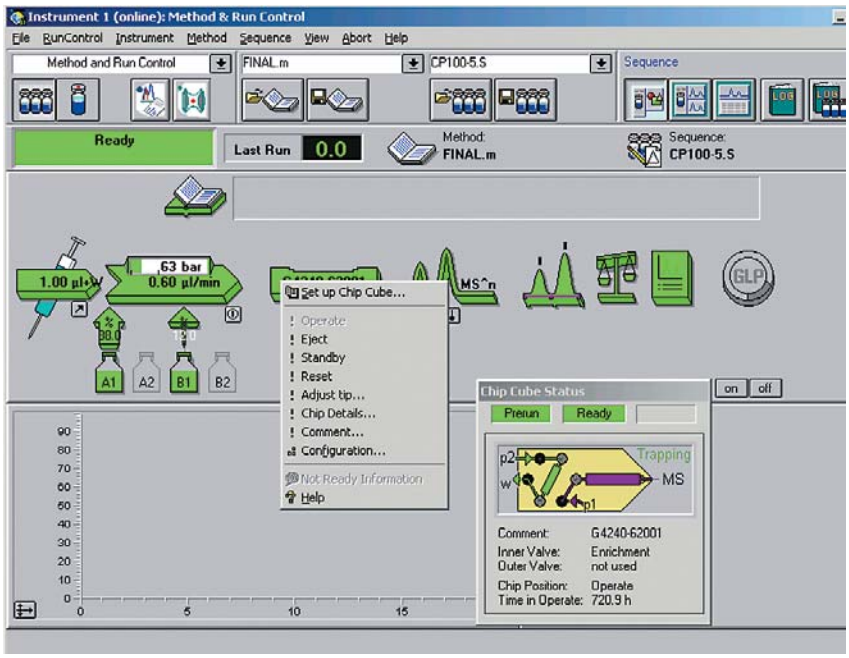
The HPLC-Chip Cube interface contains a highly sophisticated new multiport microvalve with a unique rotor-in-rotor design. This new microvalve uses a fixed stator with dual concentric rotors to deliver the solvents and sample to the different layers of the HPLC-Chip. Each rotor can operate independently of each other and can rotate a full 360 degrees in 1-degree steps. The number of possible connections that can be achieved is almost inexhaustible, ensuring compatibility with current HPLC-Chips and guarantees compatibility with future multi-layered HPLC-Chip designs that will incorporate features such as multidimensional columns and on-chip chemistries.



The inert multiport microvalve contains a unique rotor in rotor design that allows fluid delivery to even the most complex HPLC-Chip designs. The microvalve docks directly onto the HPLC-Chip for leak tight zero dead volume connections.

ChemStation operation for easy integration and control

The HPLC-Chip Cube MS interface is a standard module within the Agilent 1100 Series LC system and is fully integrated with and controlled from the Agilent ChemStation software. Loading or replacement of the chip is simple and can be completed in a few seconds with a simple mouse click.



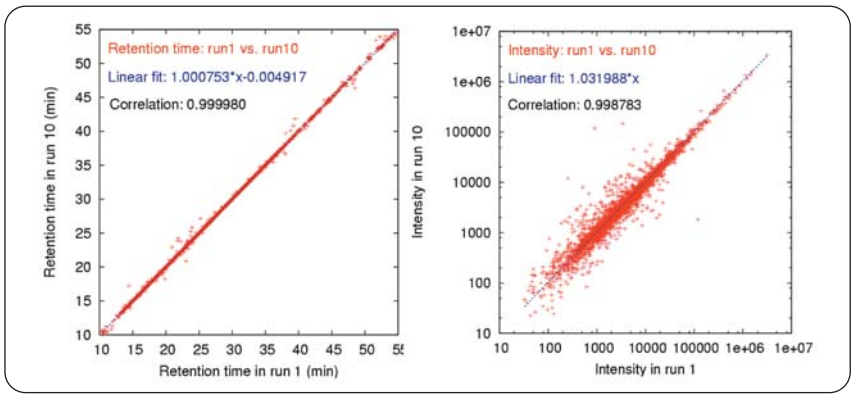
The HPLC-Chip Cube status window displays information on chip type, position and time in operation. The chip diagram highlights current run status, valve position and flow path. The pull-down menu allows quick and easy access to all HPLC-Chip Cube commands.

Take advantage of the HPLC-Chip/MS system and make nanoflow LC/MS the workhorse for your high sensitivity LC/MS analysis needs. Combining the Agilent HPLC-Chip/MS with the Agilent 1100 Series nanoflow LC and LC/MSD Trap XCT Ultra or LC/MSD TOF mass spectrometers will offer you the best possible performance for nanospray applications were sensitivity, reproducibility, reliability, robustness and ease of use are essential.

Unsurpassed chromatographic performance

Accurate nanoliter rates for superb retention time reproducibility

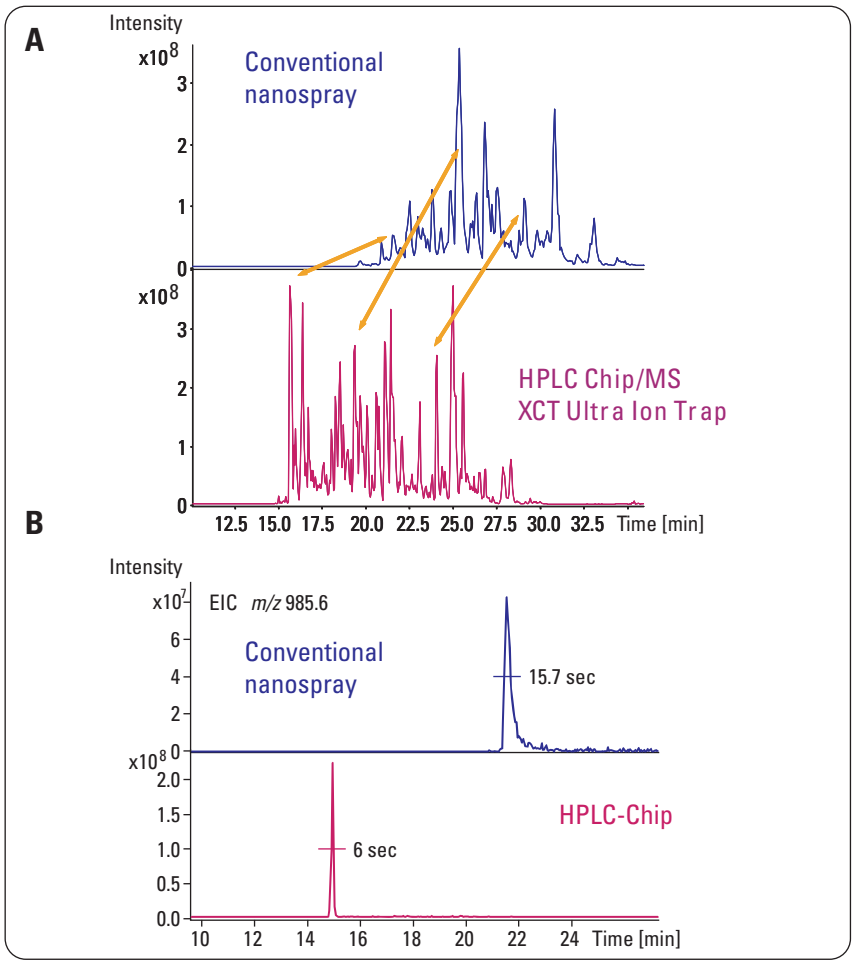
The Agilent 1100 Series nanoflow LC pump uses a unique solvent delivery technology optimized for nanoliter-per-minute flows and features electronic flow control (EFC) with active feedback that guarantees constant flow rates independent of column backpressure. This provides the extremely stable and accurate nanoliter-per-minute flow rates essential for unmatched retention-time reproducibility and stable nanospray performance. Typical performance for retention time reproducibility is less that 0.4 % RSD.



Retention time and ion intensity reproducibility: Comparison of run 1 with run 10 of a 5000 glycopetide separation using the Agilent HPLC-Chip: 43 mm x 75 µm column, 40-nL enrichment column, ZORBAX 300SB 5-µm C18. Flow of 300 nL/min, 60-minute gradient. Agilent Series 1100 nano-LC, HPLC-Chip Cube and ESI-TOF MS. Sample courtesy of Dr. Rudi Aebersold, Institute for System Biology.

Integration of columns and fittings on the HPLC-Chip for uncompromised chromatographic performance and high-sensitivity MS detection

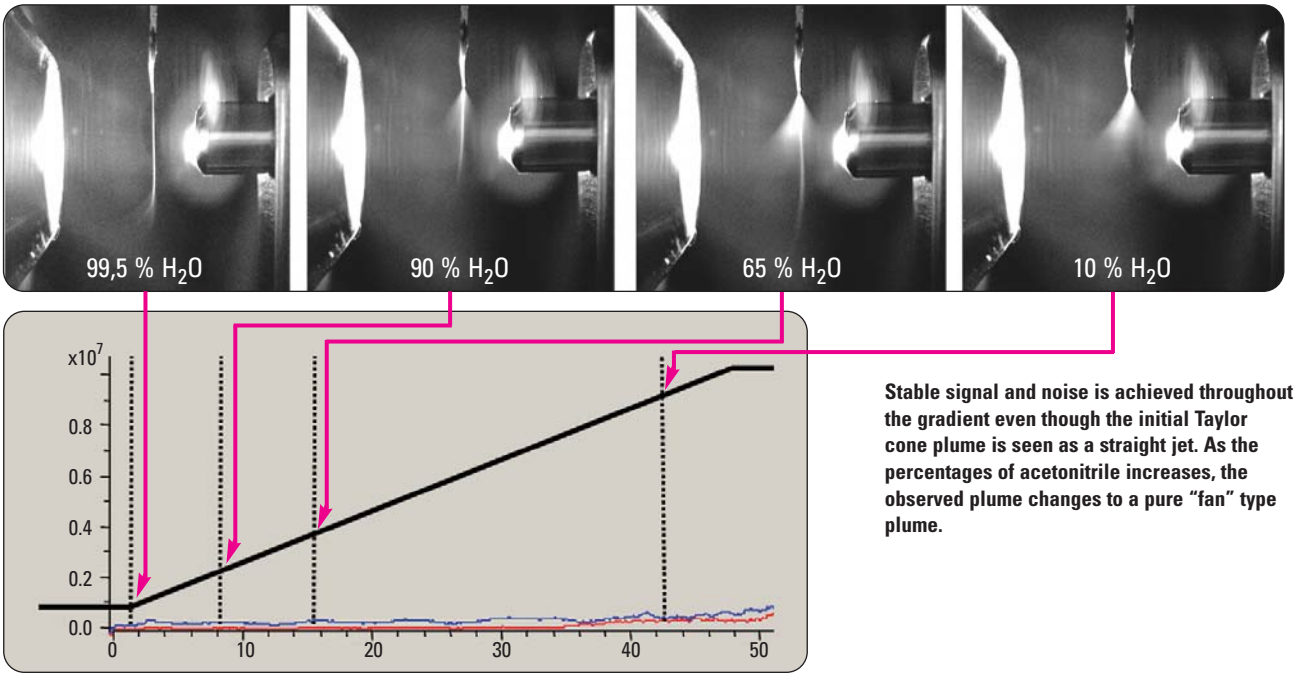
Eliminating post-column dispersion by integration of all components on the HPLC-Chip provides narrow well defined peaks. Small elution volume and enhanced peak height provide more sensitivity for concentration dependant detectors such as mass spectrometers. The microfluidic chip-based system produces chromatographic peaks that are 2 to 3 times narrower than with the conventional nanocolumn systems. This effect is particularly noticeable for the early eluting peaks. The uncompromised chromatographic performance and increased peak height dramatically enhances sensitivity of MS detection.



A. HPLC-Chip (bottom) vs. conventional nanocolumn (top): BPC of a mixture of 16 protein digests of varying concentrations.
B. EIC of *m/z* 985.6 from the 16-protein digest. HPLC-Chip (bottom), conventional nanocolumn (top).

Orthogonal dual-electrode nanospray ion source
Stable nanospray without the need for voltage optimization

Use of the Agilent orthogonal dual electrode nanospray ion source shows high signal-to-noise for all types of Taylor cone plumes when combined with the polymer HPLC-Chip. Unlike other nanospray designs, it does not require needle voltage optimization or changes during the gradient

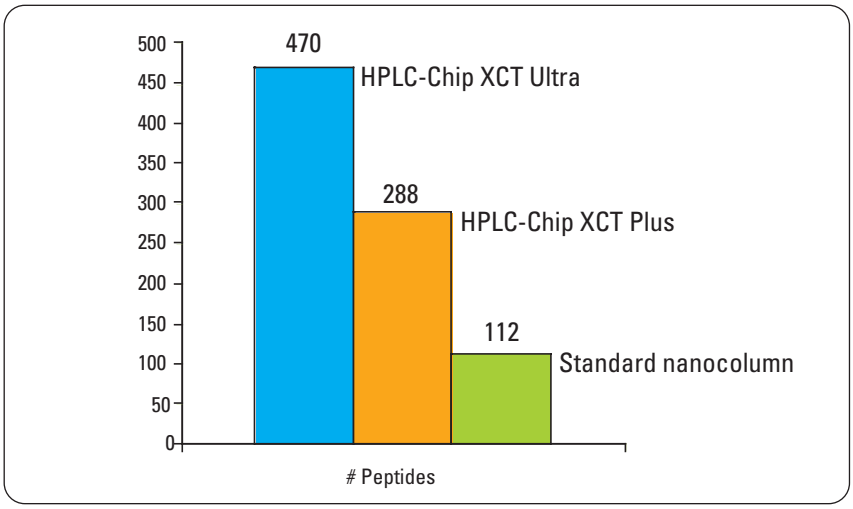


Stable signal and noise is achieved throughout the gradient even though the initial Taylor cone plume is seen as a straight jet. As the percentages of acetonitrile increases, the observed plume changes to a pure "fan" type plume.

Angiotensin I was infused with and infusion HPLC-Chip (p/n G4240-61002) at a constant flow rate of 320 nL/min. EIC traces of *m/z* 433, [M+3H]+3 (blue) and *m/z* 450-640 averaged noise/background (red). Signal to noise remained constant over the LC gradient, shown in black, from 99 % to 1 % water. Operating voltage also remained constant at 2.1 Kv throughout the gradient and did not require adjustment or programmed modifications.

Identification and quantitation of more compounds in complex samples

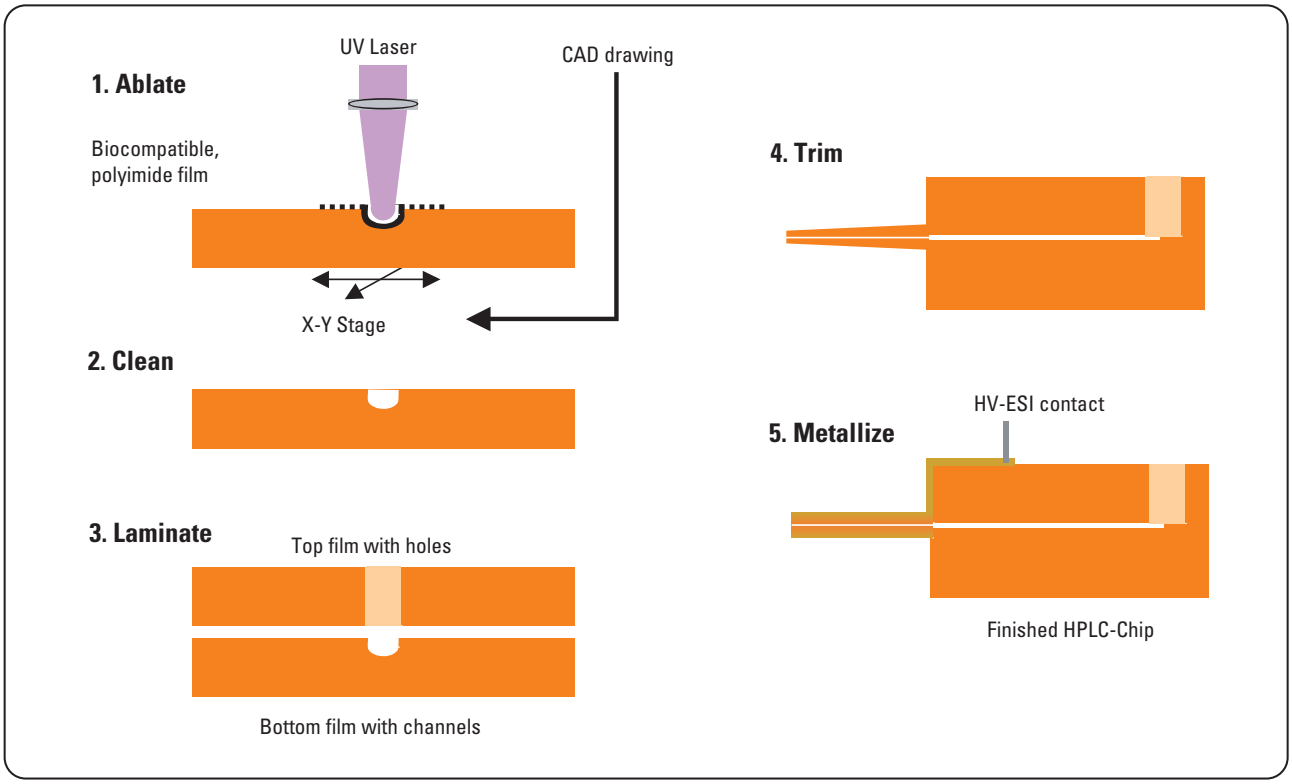
The fast scan speeds and enhanced data acquisition rates of the new LC/MSD Trap XCT Ultra system allows you to acquire more scans from the narrow peaks obtained with the HPLC-Chip. The LC/MSD Trap XCT Ultra starts with superior sensitivity and adds a new high-performance data acquisition subsystem that triples the number of scans acquired at a given scan speed. More scans increases the identification of lower-abundance compounds in the presence of co-eluting higher abundance compounds. It also improves quantitative accuracy, which can aid in biomarker discovery and small molecule analysis.



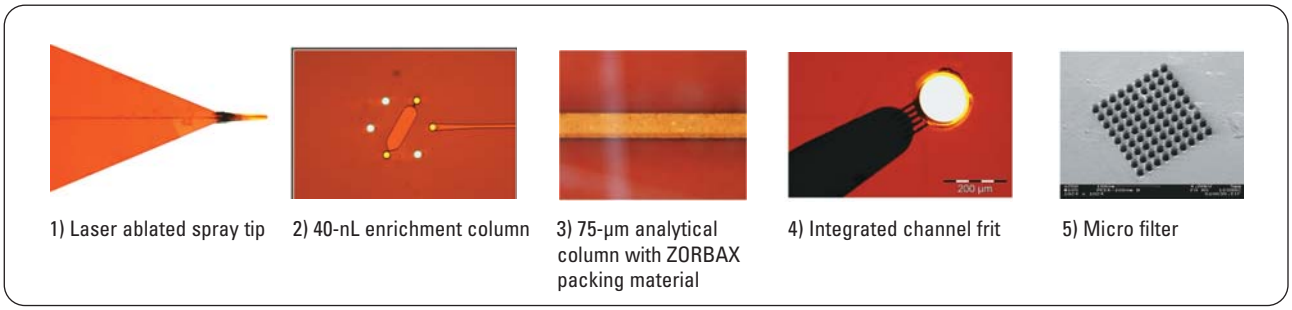
Combine results from SCX salt fractions from immunodepleted serum sample. Agilent HPLC-Chip: 43 mm x 75 µm column, 40 nL-enrichment column, ZORBAX 300SB 5 µm C18. Flow of 300 nL/min, 60-minute gradient. A 319 % increase in peptide identification was achieved with HPLC-Chip LC/MSD Trap XCT Ultra combination compared to the standard nanocolumn.

Flexible, scalable HPLC-Chip manufacturing process

Laser ablation of the biocompatible polyimide film is used to create surface structures required for the HPLC-Chip layout. This makes it possible to create chips with a wide variety of features and functionality. Lamination of several polyimide layers together allows the creation of multi-function chips. Component integration reduces the complexity and enhances the robustness of multifunction applications such as multidimensional nanospray LC/MS.



HPLC-Chip manufacturing: 1) The laser creates the microstructures directly on the polyimide surface. 2) The surface is cleaned. 3) One or several layers of polyimide containing different features such as analytical columns, filters and fluid access holes are laminated together. 4) The chip is then trimmed and the electrospray tip is directly laser ablated on the chip. 5) In the final step, the surface is metallized for high voltage electrospray contact.



1) Laser ablated spray tip

2) 40-nL enrichment column

3) 75-µm analytical column with ZORBAX packing material

4) Integrated channel frit

5) Micro filter

Surface structures required for HPLC-Chip.

Specifications: HPLC-Chip Cube MS Interface (G4240A)

Type	Specifications
Weight	14 kg (31 lbs)
Dimensions (height x width x depth)	349 x 298 x 359 mm (13,7 x 11,7 x 14,1 inches)
Line Voltage	100 – 240 VAC, +/- 10 %
Line frequency	50 or 60 Hz, +/- 5 %
Power consumption (apparent power)	300 VA
Power consumption (active power)	80 VA
Ambient operating temperature	5 – 40 °C
Ambient non-operating temperature	-40 to 70 °C
Humidity	< 80 % at 40 °C
Operating altitude	Up to 2000 m (6500 ft)
Non-operating altitude	Up to 4600 m (14950 ft)
Safety standards IEC, CSA, UL	Installation Category II, Pollution Degree 2
Materials in use	SST, Fused silica, ceramic (valve stator/rotor) microvalve
Microvalve	Stator-rotor design with dual concentric multiport rotor.
Microvalve rotor movement	360° rotation for both rotors. Inner rotor and outer rotor rotate simultaneously in the same direction or in opposite directions in 1-degree increments.
MS tuning and calibration	Dedicated stator port connection from the HPLC-Chip Cube to the Ion Trap MS calibrant syringe pump for delivery of the tuning mix to the Ion Trap MS with the MS Calibration and Diagnostic chip (p/n G4240-61001)
HPLC-Chip/MS software control	HPLC-Chip /MS interface control and automatic chip loading, ejection and diagnostic functions directly from ChemStation software (revision B01.03 or higher required).
Nanospray source	Fully integrated enclosed spray chamber and orthogonal dual electrode nanospray ion source.
Spray visualization	Integrated camera and light source directly in spray chamber. Video output to external monochrome monitor.
Connections to HPLC-Chip and flow switching	Automatic hydraulic connections to HPLC-Chip with integrated stator-rotor microvalve. Automatic electrical connections with imbedded contact pins. Computer controlled dual concentric rotor and stator dock on to the chip and automatically aligns the flow path from the nano-LC and autosampler to the port on the chip surface. Rotor movement on chip surface allows rapid flow switching.
Tip positioning	Nanospray tip automatically positioned at pre-defined optimized X/Y/Z coordinates orthogonal to MS source. Manual adjustment via software control. Pre-defined or manually selected tip position coordinates stored in HPLC-Chip RF tag allowing automatic re-positioning of chip spray tip to last used position.

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