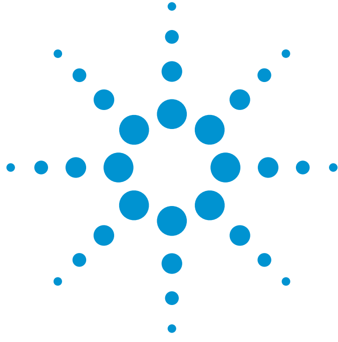

The Agilent Nanodapter for Discovery Proteomics Using Nanoflow LC/MS

Technical Overview

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Introduction

Liquid chromatography mass spectrometry (LC/MS) is commonly used for protein identification, profiling, and quantitation. While proteomics research historically has used nanospray LC/MS to achieve maximum analytical sensitivity with limited sample amounts, Jet Stream proteomics using iFunnel technology permits the use of standard flow LC/MS[1]. For discovery workflows, Jet Stream proteomics most commonly uses an Agilent 1290 Infinity LC system interfaced to an Agilent 6550 iFunnel Q-TOF mass spectrometer using the Agilent Jet Stream Source. This LC/MS system provides excellent analytical sensitivity and robustness for peptide analysis in complex matrices with 2.1-mm id columns at a standard flow rate. However, when the sample amount is limited, nanoflow LC/MS provides the maximum analytical sensitivity. The Agilent Infinity UHPLC Nanodapter can quickly convert the Jet Stream proteomics UHPLC system to nanoflow UHPLC, which, when interfaced to the Agilent G1992A nanoESI source, provides high sensitivity for discovery proteomics[2].

Agilent Infinity UHPLC Nanodapter

The Infinity UHPLC Nanodapter adapts an Agilent 1290 Infinity II binary pump to precisely deliver flow rates from 100 to 1,000 nL/min for separations using 75 µm to 150 µm id columns at pressures up to 1,000 bar (Figure 1). The in-line flow sensor provides continuous monitoring of the column flow rate. This solution is compatible with a wide range of nanoflow LC columns, facilitating optimal performance for a variety of samples.

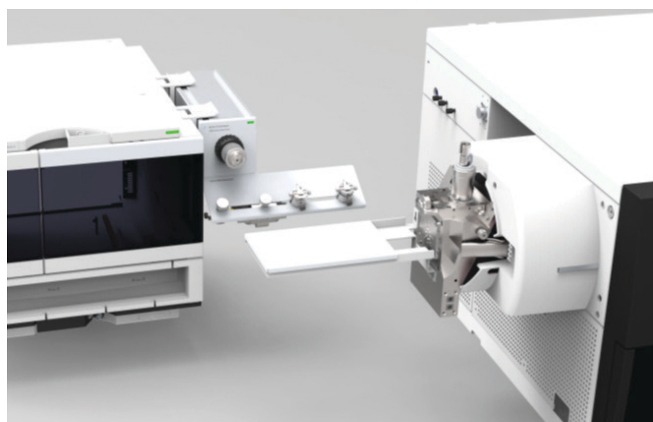

Figure 1. Agilent Infinity UHPLC Nanodapter.

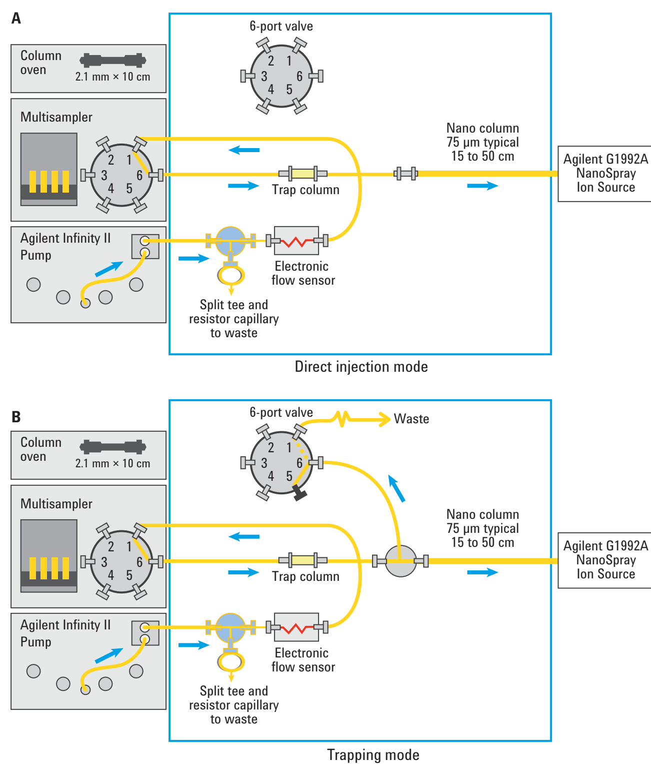

Figure 2. Agilent UHPLC Nanodapter flexible configurations.

The nanoflow LC system is interfaced to the mass spectrometer using the Agilent G1992A nanoESI source, which is flexible, easy to configure, and supports columns with integral and separate spray needles. The electrospray emitter can be positioned orthogonally to the mass spectrometer inlet, reducing the sampling of background contaminants, or in-line to the inlet, increasing sampling efficiency.

The Nanodapter provides the ultimate flexibility as both standard flow LC and nanoflow LC are supported, and changing between modes is very fast (<30 minutes). The divert valve provided allows experienced users to configure the LC system in different modes to fit method requirements. For example, the direct injection mode gives the best results for hydrophilic peptides, while the trapping mode is much faster for loading large sample volumes (Figure 2).

Discovery Proteomics with Nanoflow LC/MS

In discovery proteomics, data-dependent acquisition using nanoflow LC/MS is the most common approach. To evaluate system performance, the Nanodapter coupled with a 1290 Infinity II binary pump was interfaced to a G1992A nanoESI source, and the Agilent 6550 iFunnel Q-TOF for protein identification. A study performed in direct injection mode using a human K562 cell extract digest standard (Promega) demonstrated excellent performance using this nanoflow LC/MS platform. A 75 µm × 25 cm C18 column coupled to a 2 cm guard column was used for peptide separation (Table 1). Human K562 cell extract digest (1 µg) was analyzed using a 120-minute LC gradient and data-dependent acquisition (Tables 1 and 2).

Table 1. Liquid chromatography method setup.

LC Conditions		
Nanodapter configuration	Direct injection mode	
Guard column	Thermo Acclaim PepMap, 75 µm × 2 cm	
Analytical column	Thermo Acclaim PepMap, 75 µm × 25 cm	
Column temperature	60 °C	
Injection volume	1 µL	
Autosampler temperature	4 °C	
Mobile phase	A) H_2O, 0.1 % formic acid B) 90 % Acetonitrile, 0.1 % formic acid	
Flow rate	0.095 mL/min primary flow Primary pump pressure ~400 bar ~320 nL/min on-column flow rate	
Gradient	Time (min)	%B
	0.0	3
	59.7	18
	78.6	25
	110.0	37
	115.0	70
	117.0	70
	120.0	3
Stop time	130 minutes	

Table 2. Agilent 6550 iFunnel Q-TOF method setup.

Parameter	Value
Sprayer needle	New objective noncoated needle (20 µm id, 10 µm tip id, 5 cm length), orthogonally positioned
Gas temperature	200 °C
Drying gas flow	11 L/min
Acquisition mode	Extended Dynamic Range (2 GHz) mode 100–1,700 mass range High Sensitivity
Mass range	*m/z* 300–1,700
Acquisition rate/time	8 spectra/sec
Auto MS/MS range	*m/z* 50–1,700
MS/MS Acquisition rate/time	3 spectra/sec
Isolation width	Narrow (~1.3 amu)
Max precursors/cycle	20
Threshold for MS/MS	2,000 counts and 0.01
Dynamic exclusion	On; 1 repeat then exclude for 0.2 minutes
Precursor abundance-based scan speed	Yes
Target (counts/spectrum)	25,000
Use MS/MS accumulation time limit	Yes
Use dynamic precursor rejection	Yes
Purity	100 % stringency, 30 % cutoff
Isotope model	Peptides
Sort precursors	By abundance only; +2, +3, >+3
Default delta *m/z*	15 ppm

Figure 3 shows that excellent chromatographic separation and reproducibility were achieved with triplicate analysis. Extracted ion chromatograms (EICs) were evaluated for all peptide precursors identified from heat shock protein HSP 90-beta. Results showed good peak symmetry and a median 13-second peak width at half maximum height (PWHM), which demonstrated excellent chromatographic performance (Figure 4). A total of 20,735 unique peptide sequences and 2,833 unique proteins were identified across the triplicate LC/MS runs, with an average of 16,025 unique peptides and 2,437 unique proteins identified in each analysis, demonstrating excellent sensitivity and reproducibility (Figure 5).

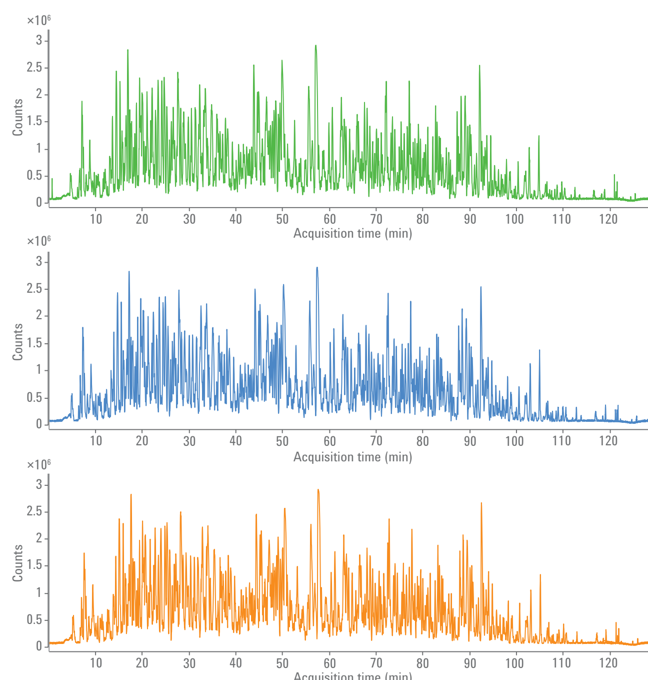

Figure 3. Base peak chromatograms of a triplicate analysis of human cell extract digest with a 120-minute gradient.

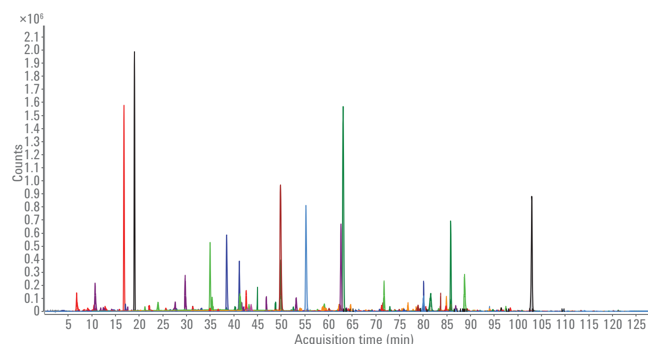

Figure 4. Overlaid EICs for all peptide precursors identified from heat shock protein HSP 90-beta.

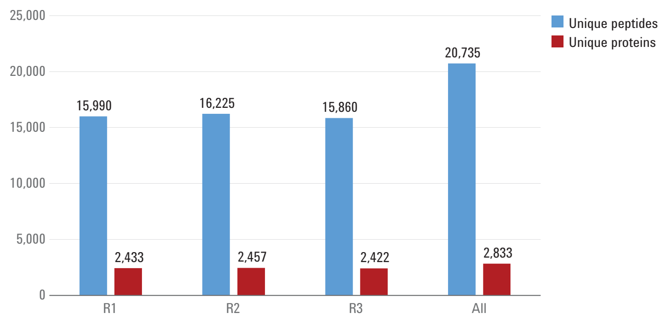

Figure 5. Triplicate analysis of Human K562 cell extract digest (1 µg on-column) analyzed on a 75 µm × 25 cm column with a 120-minute gradient. Protein database searching was done using Agilent Spectrum Mill Software, and peptide spectral matches were validated using a 1.2 % peptide false discovery rate (FDR) followed by 1 % protein FDR filter.

Conclusion

The flexible, easy-to-configure Agilent Infinity UHPLC Nanodapter is designed to provide the ultimate flexibility for experienced users. The combination of the Nanodapter with an Agilent 1290 Infinity binary pump allows a single UHPLC system for both standard flow and nanoflow LC analysis. The design easily accommodates columns of different lengths and inner diameters to meet user method requirements. Where sample amounts are limited, nanoflow LC/MS with the 1290 Infinity binary pump, Infinity UHPLC Nanodapter, Agilent G1992A nanoESI source, and the Agilent 6550 Q-TOF offers the ultimate sensitivity. The excellent performance of the nanoflow LC/MS has been demonstrated by a discovery proteomic analysis of human cell extract digest.

References

1. Jet Stream Proteomics for Sensitive and Robust Standard Flow LC/MS, *Agilent Technologies*, publication number 5991-5687EN.

2. Maximize Nanoflow LC/MS Performance Using the Flexible, Easy-to-configure Agilent nanoESI Source, *Agilent Technologies*, publication number 5991-1041EN.

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