A semi-automated AssayMAP Bravo protocol for robust Evotip loading and high-throughput analysis on the Evosep One

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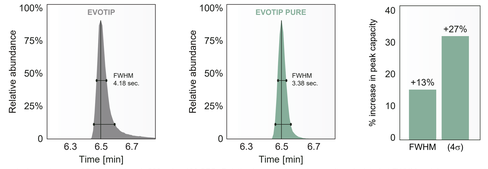
Highlights

- Development of two robust and high-throughput methods for Evotip loading using the Agilent AssapMAP Bravo
- Evosep One/Agilent 6495C combination represents a stable platform for targeted applications.
- Methods are within clinical demands (15-18 min) and analytical standard (6.3-8.6% CVs).

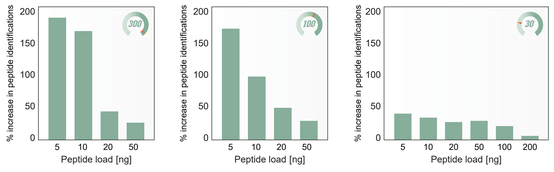
Better chromatography with Evotip Pure

Integration of elution and liquid chromatography

Evotip Pure™ is a new generation Evotip developed for high performance. It provides better chromatographic performance and ensures improved results and consistency over time. The improved peak definition and peak width will significantly increase peak capacity and ultimately lead to better performance.



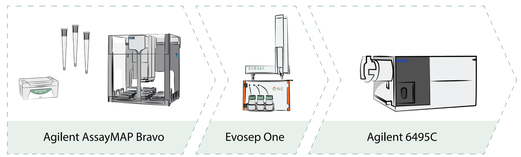
Extracted base peak chromatogram of BSA peptide m/z 582 using 100 SPD. Relative increase in peak capacity calculated at FWHM and 4σ compared to the old Evotips.



Relative increase in IDs with the Evotip Pure compared to the old Evotip using the 300, 100 and 30 SPD methods.

Simplifying workflows by speeding up sample loading

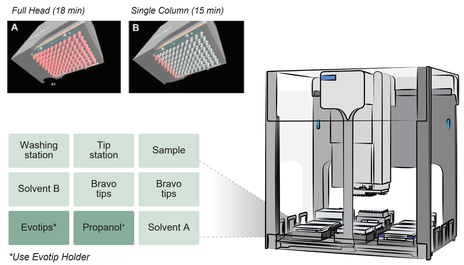
Here we introduce the use of the AssayMAP Bravo for semi-automated sample loading of Evotips. We loaded 200 fmol BSA in 96 replicates with two different AssayMAP protocols and compared to 96 samples loaded manually with the standard loading protocol. All 288 samples were measured with Evosep-MRM on a Agilent 6495C.



Chromatographic performance

Two protocols designed for Evotips

We developed two protocols for transferring samples from a 96 well sample plate to a box of Evotips. The first method ('full head') involved simultaneous transfer of a full set of samples from individual positions in a 96 well plate to Evotips. The second method ('single column') was designed to support user flexibility and allows for transfer of 8 samples (or a single column from a 96 well plate) at a time.



*Use Evotip Holder
*Use Equilibration Holder

Loading protocol for Evotip Pure

RINSE
Wash dry Evotips with 20 µl Solvent B and centrifuge at 800 g for 60 s.

CONDITION
Soak in Propanol until all the Evotips are pale white.

EQUILIBRATE
Equilibrate soaked Evotips with 20 µl Solvent A and centrifuge at 800 g for 60 s.

LOAD
Load samples on wet Evotips (20 µl in Solvent A) and centrifuge at 800 g for 60 s.

WASH
Wash Evotips with 20 µl Solvent A and centrifuge at 800 g for 60 s.

WET
Transfer 100 µl Solvent A and centrifuge Evotips at 800 g for 10 s to keep them wet.

Layout of AssayMap Bravo deck for semi-automated loading protocols using the 'full head' or the 'single column' protocol.

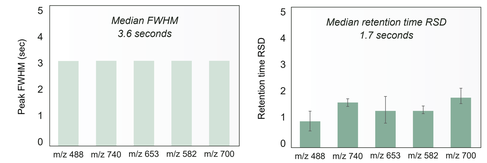
Availability of AssayMAP Bravo loading protocols

Two holders are required to run the protocols; an 'Evotip' holder and an 'Equilibration' holder. The corresponding 3D print files can be found online at www.evosep.com/support/automation. The actual protocols to load on the AssayMAP Bravo can also be found here.

Excellent chromatographic performance

We extracted data for five different BSA peptides eluting across the 100 SPD method to assess the stability of retention times (RT) across 288 injections. This resulted in a median RT relative standard deviation (RSD) of 1.7 seconds. The median full peak width at half maximum (FWHM) is consistent at 3.6 seconds, which reflects the high reproducibility supported by Evotip disposable trap columns as well as the Evosep system itself.

Peptide precursor	m/z
TCVADESHAGCEK	488.53
LVNELTEFAK	582.32
HLVDEPQNLIK	653.36
TVMENFVAFVDK	700.35
LGEYGFQNALIVR	740.40

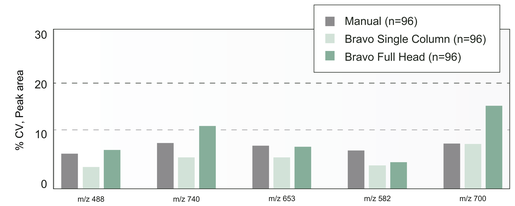


Chromatographic performance across the 288 injections with the 100 SPD method.

Robustness of quantification

Reproducible peak areas with all three methods

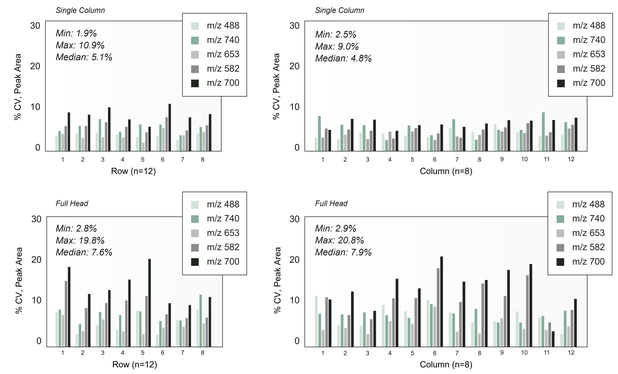
The reproducibility associated with manual loading was equally impressive compared to the two semi-automated protocols with coefficient of variation (CV) of 8.6% against 6.3% and 8.5% for the two Bravo protocols.



Reproducibility of peak areas for all detected peptides for each method (n=96).

A detailed look at the two Bravo loading protocols

The reproducibility of peak area is not influenced by loading position indicated by peak area CV%. The 'full head' protocol provides slightly higher CVs than the 'single head' protocol



Reproducibility of peak areas across rows and columns with both AssayMAP Bravo loading protocols.