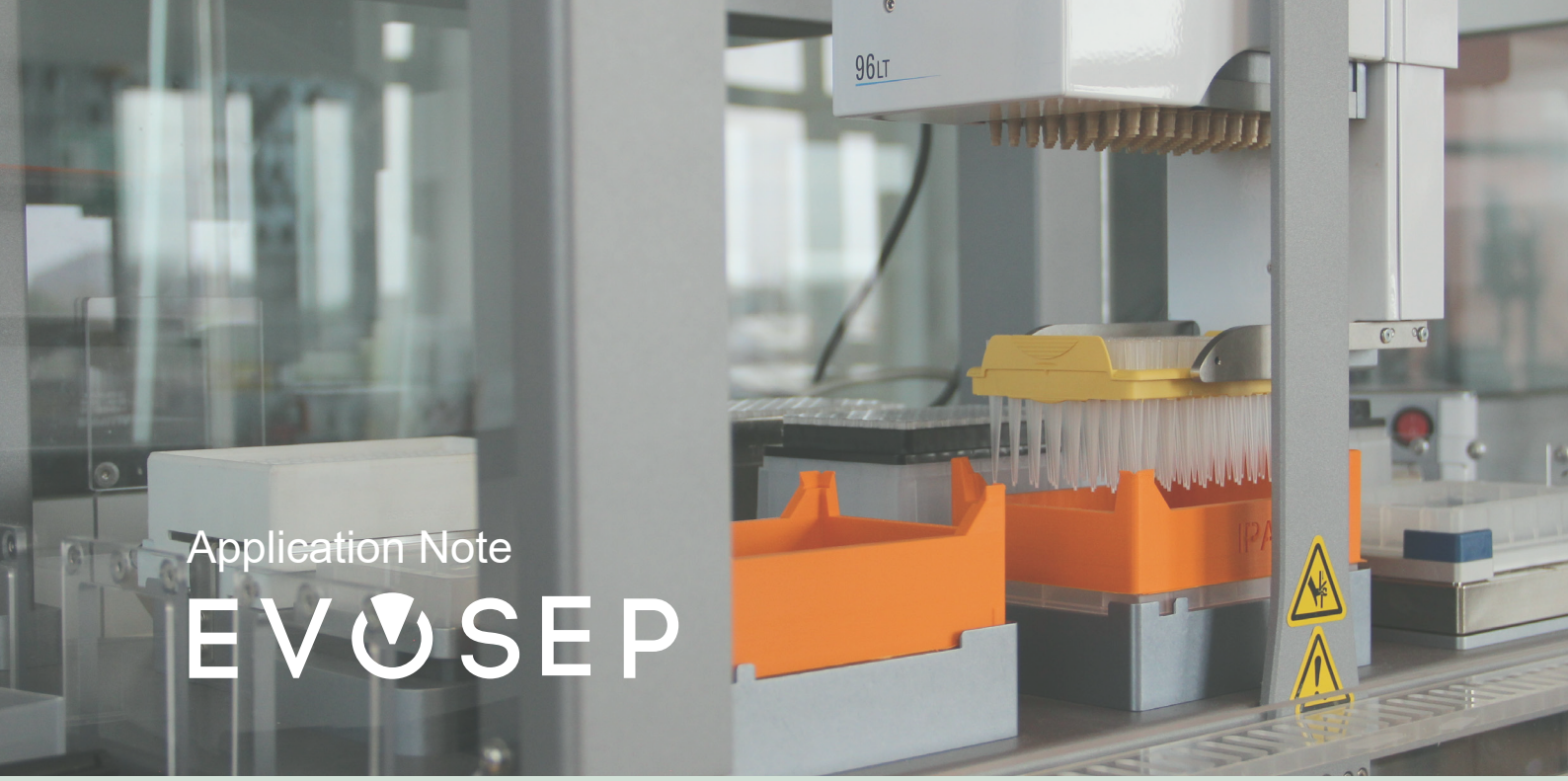

Application Note

EVOSEP

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

1. Introduction

Automation, robotics, and new technologies are transforming the field of proteomics as they help overcome hurdles associated with precision, throughput, and cost in large-scale studies. Indeed, with increasingly large numbers of samples being included in proteomics research, demand is growing for automated processes in the entire workflow from sample preparation to data analysis.

The Agilent AssayMAP Bravo platform was engineered to automate sample preparation ahead of LC-MS/MS. It incorporates a liquid handler with 96 probe syringes that allow precise positive displacement flow control and flexible customizable protocols with user friendly interfaces. It minimizes hands on time and maximises throughput allowing researchers to focus on other tasks.

The Evosep One LC-system was designed to enable robust and reproducible high-throughput analysis of large cohorts of samples. Traditional approaches require a lot of human interaction, and these step wise protocols are tedious and error prone. Recognizing the need for

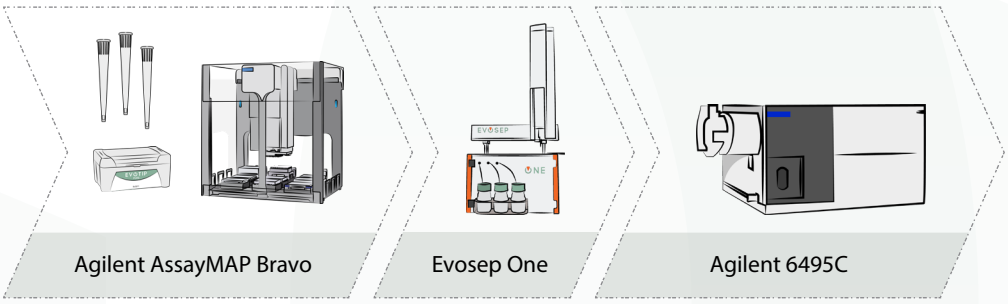

Figure 1: Workflow for semi-automated Evotip loading using the Agilent AssapMAP Bravo.

standardization and throughput, the Evotip was designed to integrate sample loading with desalting and liquid chromatography, thereby improving efficiency and recovery associated with sample purification and loading ahead of MS analysis. Here we introduce the use of the AssayMAP Bravo for semi-automated sample loading of Evotips. We developed two protocols for transferring samples from a 96 well sample plate to a box of Evotips. The first method involved simultaneous transfer of a full set of samples from individual positions in a 96 well plate to Evotips. The second method 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.

2. Method details

Bovine Serum Albumin (BSA) digest was purchased from Bruker LabScape Daltonics (8217498) and diluted to a final concentration of 200 fmol. For the 'full head' protocol, 50 µl of sample were transferred to all wells in a 96 well plate (Thermo Scientific, 10445543). For the 'single column' protocol, 400 µl were transferred to each of 8 wells in the first column in a conical bottom well plate (Thermo Scientific, 249946). The sample plate was placed in position 3. 80 ml solvent A and B were transferred to reservoirs (Chromtech, 201254-100) and placed in positions 9 and 4 respectively. 80 ml of Propanol were transferred to a single-well plate (Thermo Scientific, 242811) and then fitted into the 'Equilibration' holder in position 8. Evotip Pure (Evosep, EV2011) were inserted into the 'Evotip' holder and placed in position 7. Bravo disposable tips, 250 µl (Agilent, 19477-002) were placed in racks 5 and 6. The AssayMAP Bravo was used to perform all liquid transfers and sample loading using either the 'single column' or 'full head' protocol. Manual centrifugation between each step was performed at 800 g for 1 min as described below or online at www.evosep.com/evotip

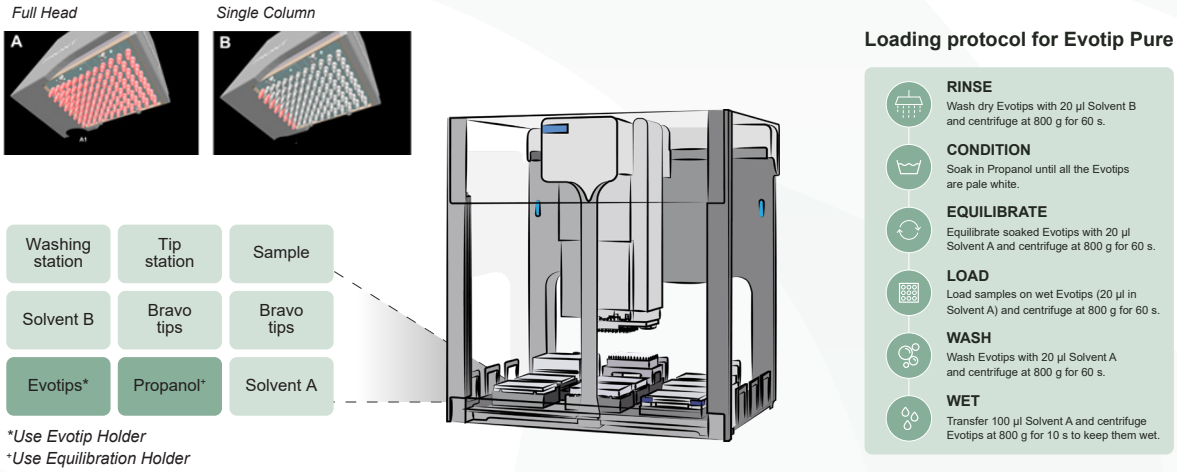

Figure 2: Layout of the AssayMAP Bravo for Evotip loading with either 'single column' or 'full head' protocol and an overview of actual steps in the protocol.

200 fmol BSA was loaded in 96 replicates with each AssayMAP Bravo loading protocol and compared to 96 samples loaded manually with the standard loading protocol. All 288 samples were measured in a randomized order with the 100 samples per day method using the EV1109 column.The Agilent 6495C mass spectrometer was operated in positive ion mode with a gas temperature at 200 °C and a drying gas flow of 11 L/min. The capillary voltage was 1750 V, the High/Low pressure RF voltage was 200/110 V and the Delta EMV was 200 V. Q1 and Q3 were set to Unit/Wide. Min and Max dwell time were set to 51.88 and 164.09 ms respectively. A cycle time of 500 ms was used for 18 MRMs. Raw data was analyzed with Skyline (21.2.0.425).

3. Robustness of quantification

We investigated the reproducibility of the two AssayMAP Bravo loading protocols by comparing the peak areas for all targeted peptides and determine if there was any analytical bias introduced across pipetting rows or columns. However, the reproducibility was not influenced by loading position indicated by peak area CV (Figure 3).

We also compared the manual loading protocol against the two AssayMAP Bravo loading protocols, which in all cases showed excellent reproducibility. The 96 samples loaded manually provided a median peak area CV of 8.6%, whereas the AssayMAP Bravo protocol with the 'single column' resulted in a median peak area CV of 6.3%. This is slightly higher at 8.5% when the 'full head' protocol is used (Figure 4).Thus, the 'single column' protocol provides the most reproducible dataset. There is an additional step associated with the 'full head' protocol since the BSA standard was manually pipetted into each well of a 96 well plate, which could contribute to some of the observed variability.

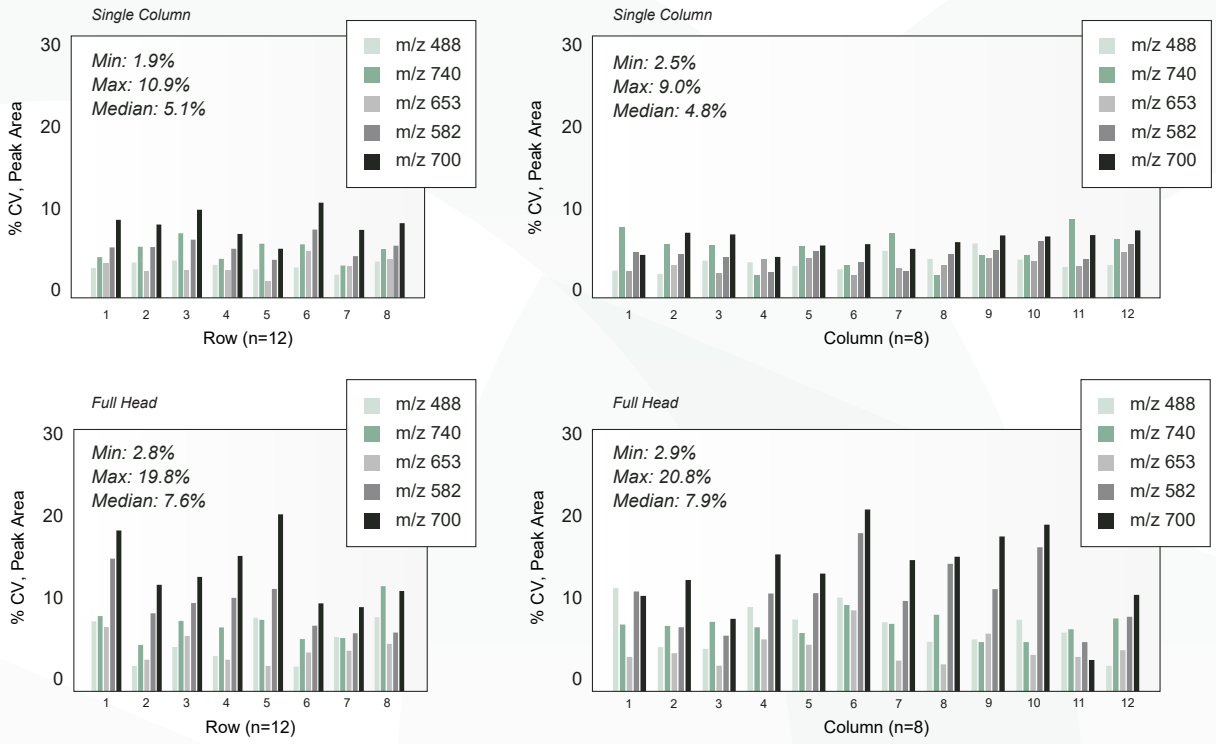

Figure 3: Reproducibility of peak areas across rows and columns with both AssayMAP Bravo loading protocols.

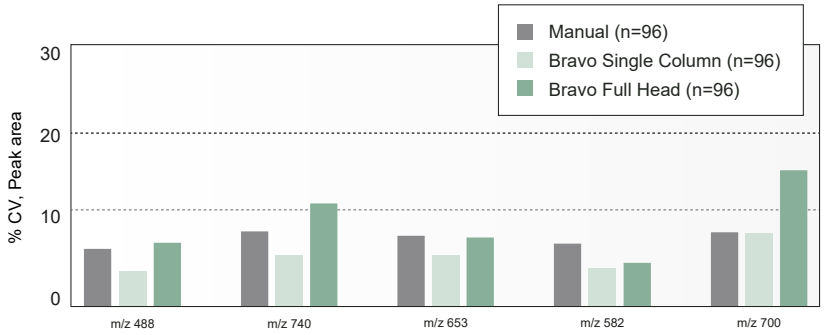

Figure 4: Reproducibility of peak areas for all detected peptides for each method (n=96).

4. Conclusion

It is important to automate as many components as possible in a preanalytical pipeline to help minimize errors, improve throughput and thereby robustness in a dataset. The data presented here highlights that the AssayMAP Bravo supports efficient and reproducible loading of samples onto Evotips with a median peak area CV of 6.3% and 8.5% when using the single column and the full head protocol respectively. This workflow can easily be combined with a range of already existing workflows on the AssayMAP Bravo. Furthermore, the Evosep One combined with the Agilent 6495C represents a stable analytical platform for targeted applications.

Availability of AssayMAP Bravo loading protocol

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.