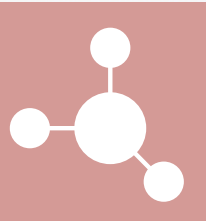


Automating Sample Preparation for High-Throughput Metabolomic Profiling

Using the MxP® Quant 500 kit and TriPlus RSH Autosampler




METABOLOMIC PROFILING

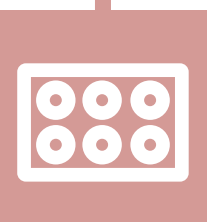
The analysis of small molecules and lipids in blood offers insights into systemic physiological and pathological states. Accurate metabolite quantification is essential to detect metabolic alterations associated with health, nutrition and disease status and therapeutic interventions, underpinning biomarker discovery and precision medicine. Achieving reliable and consistent metabolite quantification in complex biofluids such as blood serum and plasma **requires standardized sample handling and analytical workflows.**


AT A GLANCE

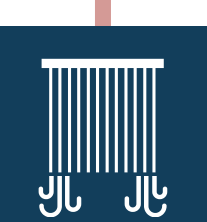
Targeted metabolomics kits offer standardized quantification of circulating metabolites (serum/plasma) for various applications, e.g., to study metabolic health and disease metabolism or to discover biomarkers. The commercially available MxP® Quant 500 kit from biocrates offers quantitative metabolomic profiling of up to 630 metabolites from 26 biochemical classes in a variety of biological matrices. However, even with standardized assays, **manual sampling handling remains a bottleneck** for large-scale studies, limiting throughput and introducing potential variability. Our ongoing work focuses on automating the MxP® Quant 500 workflow using a TriPlus RSH Autosampler to improve reproducibility and throughput while reducing personnel time. A key achievement has been the development of scripts to automate the sample addition and general liquid handling steps. **This automation minimizes hands-on time and reduces human error, leading to improved precision.** While full workflow validation is pending, future development aims to automate online LC-MS/MS and/or FIA-MS/MS injections and plate transfers, paving the way for a near-fully automated system for robust, large-scale metabolomics quantitation.

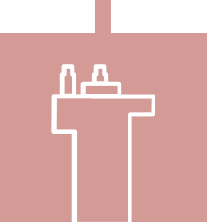
PLATE SETUP AND PREPARATION

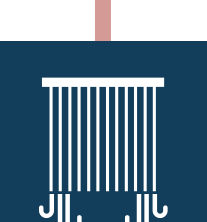
**Project design** in WebIDQ

**Sample preparation (automated)**
Pipette samples, standards and QCs and liquid handling steps, i.e., derivatization and dilutions steps.
Split extracts to generate LC and FIA plates.

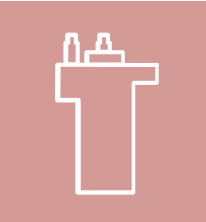
**Input** - 10 µL of plasma or serum
Using the Liquid Tool with 25 µL syringe.
After each sample transfer, wash the needle to avoid carry-over.


**Drying** - for 30min
Ideally, using a Pressure manifold for 96-well plates (later also for filtration).
Plate movement to the manifold can also be automated (optional).


**Derivatization** - Add 50 µL of PITC derivatization solution
Using the Dilutor Tool add the solution to each well. A special “wipe-off” move was created to ensure robustness and repeatability in this step.

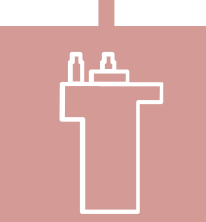
**Incubation and drying**
Incubate plate for 60 min and dry for 60 min.
Move the plate to the pressure manifold again.

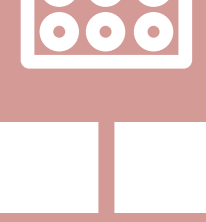
SAMPLE EXTRACTION AND ANALYSIS

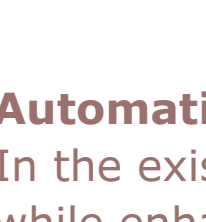
**Extraction** - Add 300 µL extraction solvent
Using the Dilutor Tool add the solution to each well.

**Shake** - for 30min
Using a 2D planar shaker.
This can also be automated using the Thermal Mixer.

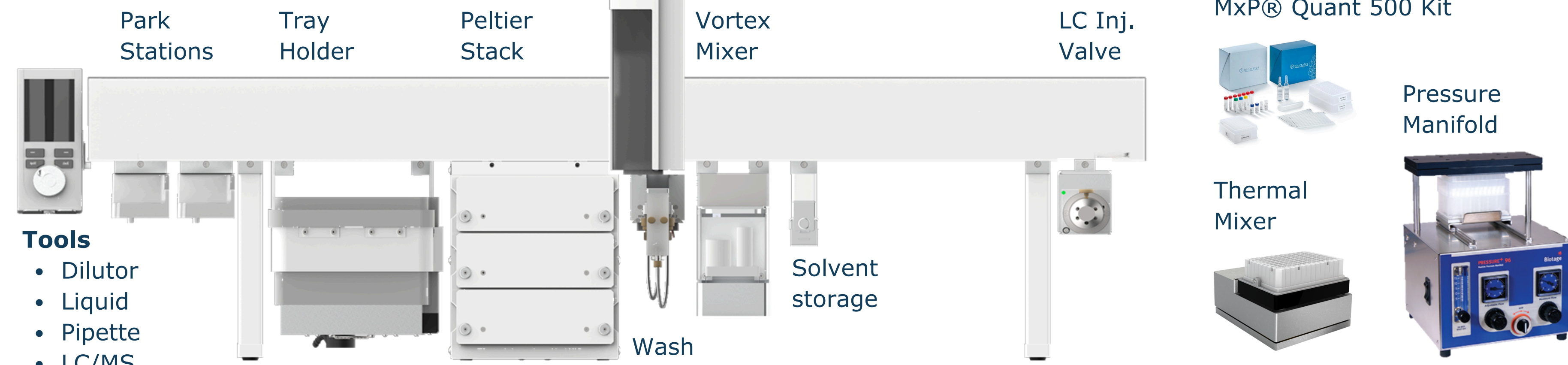
**Elution**
Pushing the extracts through the filters using the pressure manifold.
This can also be done with a centrifuge.

**Measurement plates** - LC plate and FIA plate

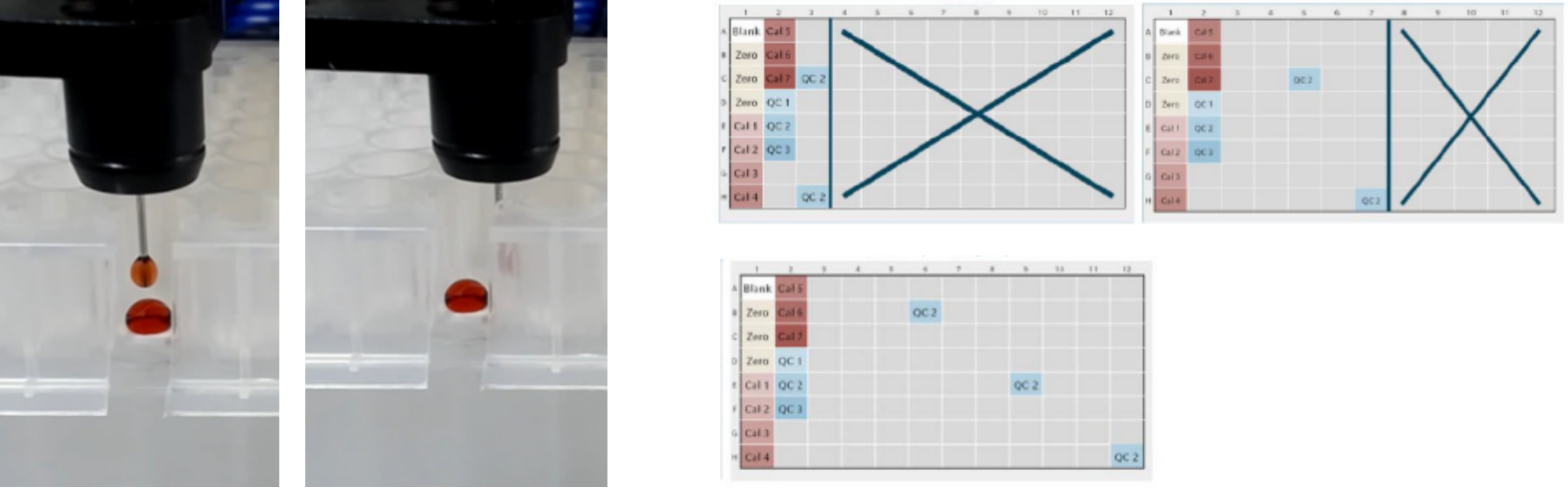
**LC plate**
Transfer 150 µL extract per well to the LC plate and dilute with 150 µL water using the Pipette Tool and Dilutor Tool.

**FIA plate**
Transfer 10 µL extract per well to the LC plate and dilute with 490 µL FIA solvent using the Pipette Tool and Dilutor Tool.

Automation Note
In the existing workflow, automated sample input and liquid handling exclude potential human errors while enhancing reproducibility. Further automation of additional steps, i.e., online injection, can lead to even fewer manual interventions.

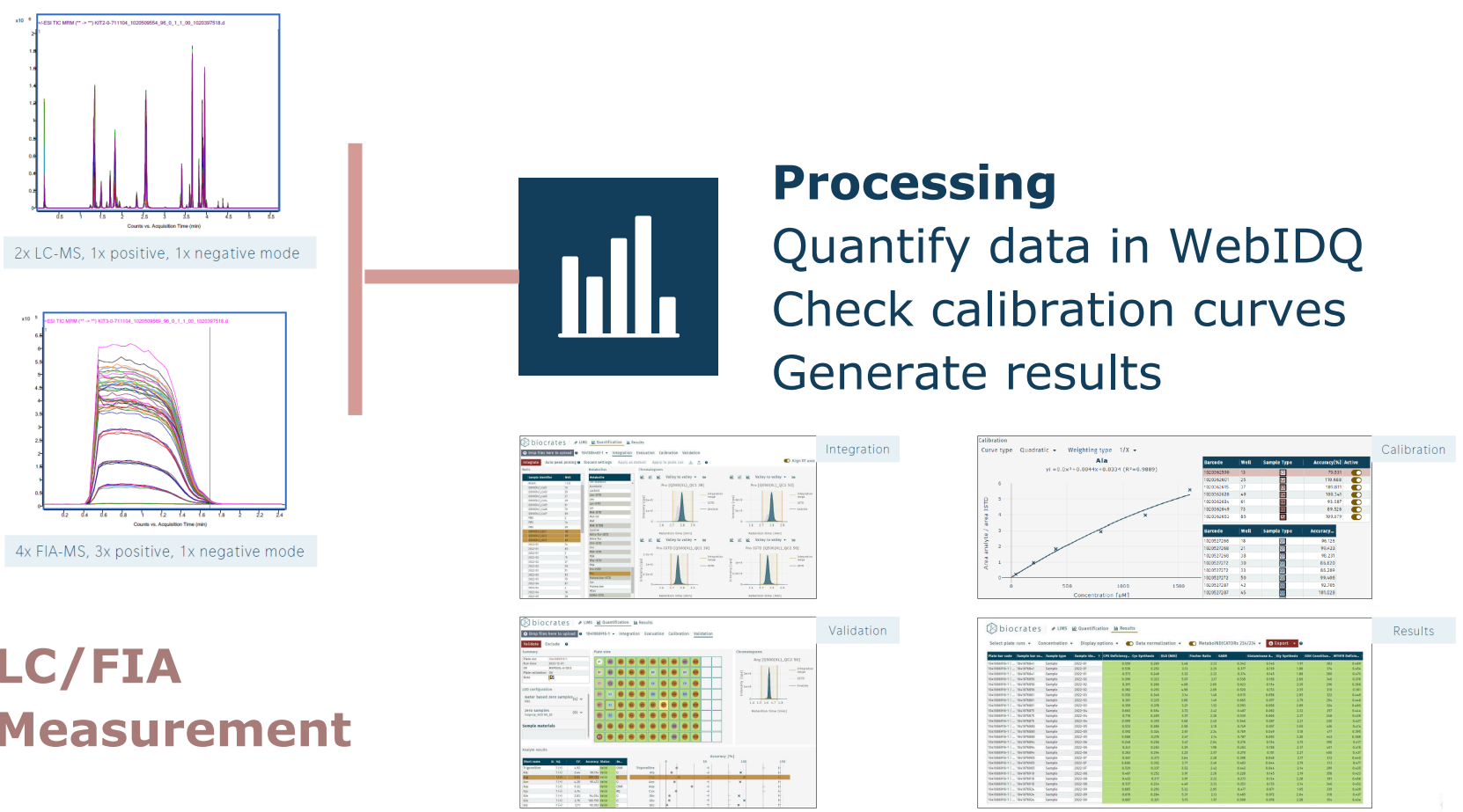


Automation



“Wipe-off” of dilutor needle in a well.


Script options: Automated transport of samples, QC and calibration standards to filter plate for Starter, Half, and Full Kits.



Processing
Quantify data in WebIDQ
Check calibration curves
Generate results

LC/FIA Measurement

Manual

**KEY ADVANTAGES**

Versatile Scripting
Adapts to Starter, Half, & Full Kits automatically.

Precision Liquid Handling
Accurate sample dispensing.

Controlled Sample Cooling
Maintains sample integrity (down to 4°C) with PAL Tray Cooler or Peltier Stack.

Direct Online Injection
Inject easily into LC/MS or FIA/MS.

Room to Grow
Start with sample dispensing and grow the automated tasks, e.g., incorporating a Thermal Mixer.