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THE MEASURE OF EXCELLENCE

Automated sample pretreatment for the analysis of synthetic cannabinoids in urine with LC-MS/MS

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Introduction

LC-MS/MS is a reliable, fast and highly sensitive technique used for multi analyte quantification of synthetic cannabinoids (SC). The analysis of the SC: JWH 018-M-N-5-OH, JWH 018-M-N-5-acid, JWH 073-M4-OH, JWH 073-M-N-B acid, JWH 081-5-OH, JWH 081-5-acid, JWH 122-5-OH, JWH 210-5-acid, JWH 250-5-OH, JWH 250-5-acid, AB-PINACA-OH, AB-PINACA-acid, AKB-48-OH, AKB-48-acid and PB-22-OH is performed for abstinence testing. Today's laboratories are challenged with delivering high standards of laboratory services with fewer resources, so full workflow automation is key to delivering highly reliable results in the required timeframe. In particular, sample pretreatment is a bottleneck requiring several tedious pipetting steps that can lead to errors. This poster describes in detail sample pretreatment automation for the analysis of synthetic cannabinoids using the HAMILTON STARlet sample pretreatment robot combined with a Sciex QTRAP 3200. Since no commercially available SC kits are available, we developed a method, making Hamilton the sole provider for this solution.

Material and Methods

HAMILTON STARlet with Autoload, 4 pipetting channels, centrifuge, CO-RE grip tools, Heater Shaker 3.0, cooling module, carrier for samples, Eppendorf Protein LoBind deep well plates, 1000µL tips, reagent troughs, Sciex QTRAP 3200.
The validation was performed on eight separate days by testing accuracy with QC (quality control) samples spiked with low concentrations of the analyte and negative controls. A comparison was carried out between manual and automated pretreatment with up to 40 spiked samples with low, intermediate and high concentrations.

Figure 1: ML STARlet equipped with a centrifuge, vacuum station, heater shaker and cooling module; further available methods are immunosuppressant, neuroleptics, antiepileptics, antidepressants and antibiotics;
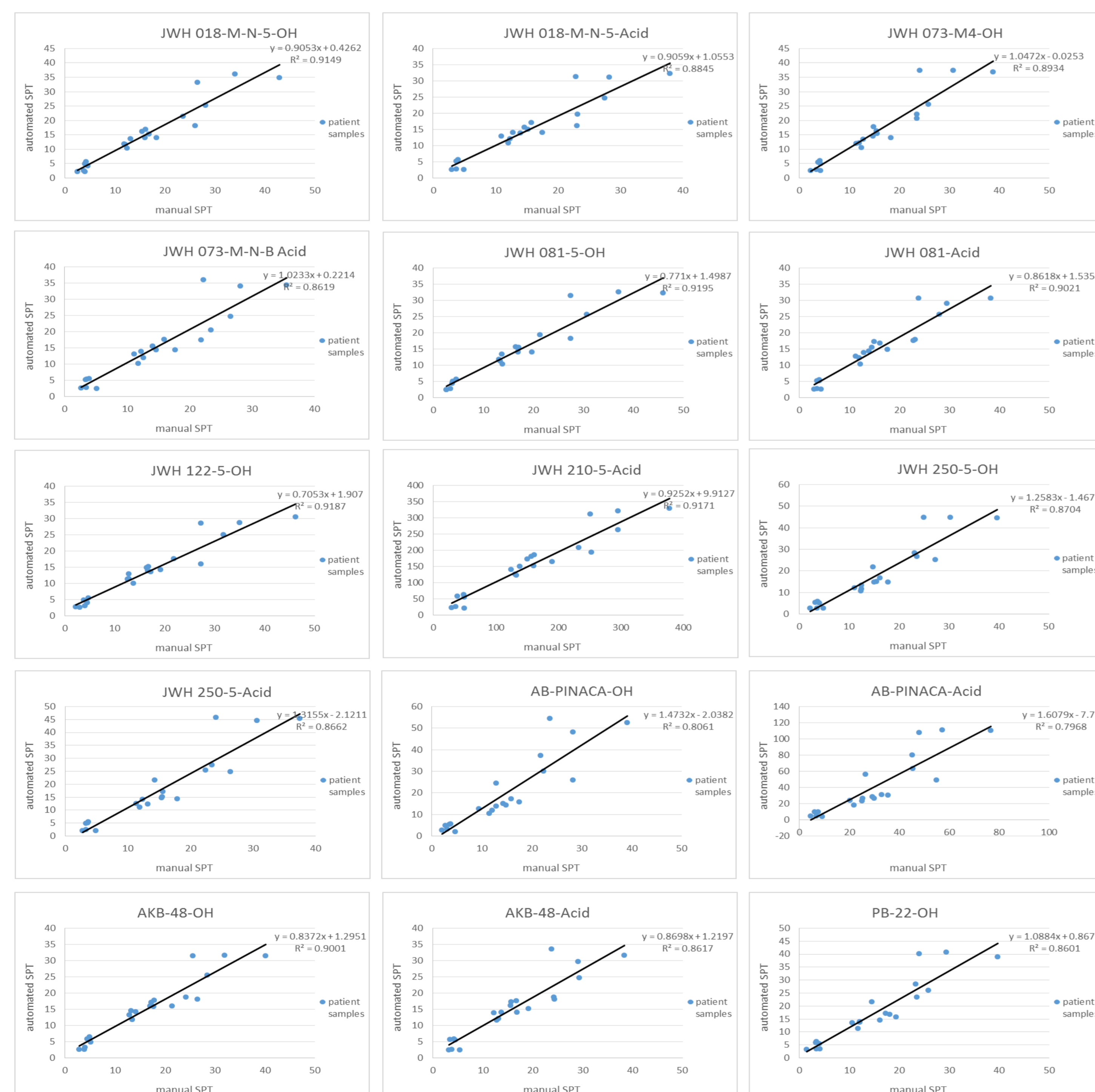

Figure 2: Workflow for the analysis of synthetic cannabinoids in urine

Figure 3: Comparison of the manual sample preparation against the automated

Results

The application was successfully developed as a walk away method; loading and unloading are the only manual steps. The automated workflow can handle up to 70 barcoded samples per run without the need of a worklist. The cooling module allows the samples to be stored in the instrument for at least 72 hours after processing and before analysis (decrease: 1.3-9.0%). It could be shown that the automated sample pretreatment with the HAMILTON STARlet performs very well with acceptable bias (0.5-14.7%) and precision (6.6-14.1%). The results correlated to those obtained with manual processing (R^2: 0.7968-0.9195). The reduced correlation coefficients are due to the use of Protein LoBind plates compared to silanized vials in manual sample pretreatment. The synthetic cannabinoids have the property to bind rather well to plastic surfaces, however the LoBind plates have the best comparability to manual sample pretreatment. The results from conventional deep well plates differed more than 70%.

Synthetic Cannabinoids (SC)	min	max
Bias [%] (< ±15%)	0.50	14.70
Imprecision [%] (< ±15%)	6.56	14.13
Decrease of analyte concentration after 72h [%]	1.3	9.0
Correlation coefficient (manual vs. automated sample pretreatment)	0.7968	0.9195

Figure 4: Summary of the analysis of synthetic cannabinoids in urine

Summary

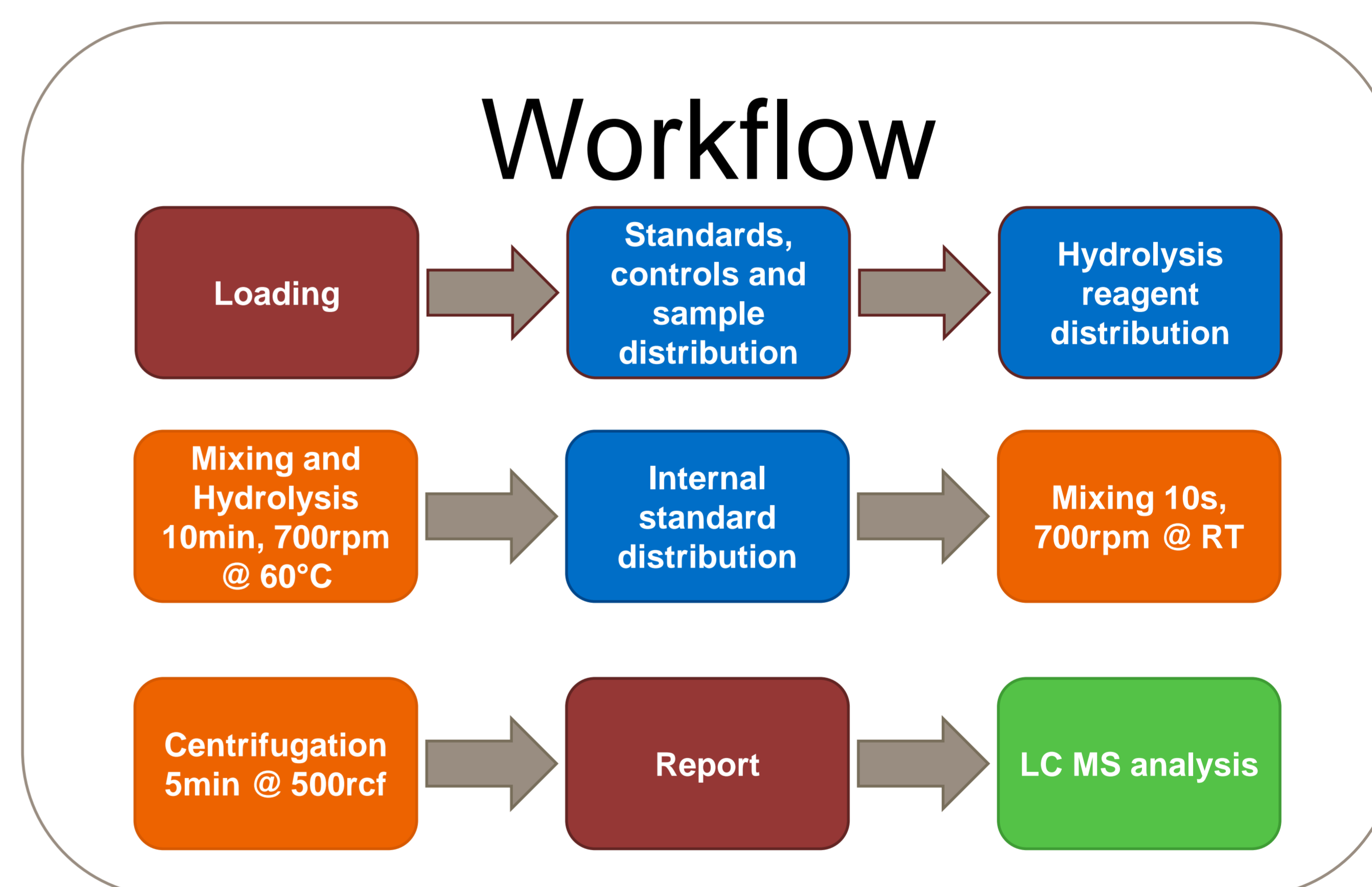With our automated method we show that sample pretreatment can be immediately implemented without the need for additional personnel.