ENHANCEMENT OF TRACEABILITY, THROUGHPUT AND ECOLOGIC FOOTPRINT OF A VITAMIN B1/ B6 LC-MS WORKFLOW VIA FLUENT® AUTOMATION.

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BACKGROUND.

Vitamins B1 (thiamine) and B6 (pyridoxine) are essential micronutrients involved in numerous metabolic processes, incl. energy production, neurotransmitter synthesis, and amino acid metabolism. Deficiencies in these vitamins can lead to a range of neurological and hematological disorders, making accurate assessment of their levels crucial for nutritional research. Quantification of Vitamin B1 and B6 typically requires analysis of plasma or whole blood samples, which can be challenging due to low endogenous concentrations and the instability of these analytes[1,2]. Automated sample preparation and analysis workflows have become increasingly important[3], enabling high-throughput, standardized, and reproducible processing of whole blood specimens. These advances help ensure reliable monitoring the Vitamin B1 and B6 status in clinical research settings.

OBJECTIVE.

This study aimed to evaluate the sample preparation and analysis of Vitamin B1 (Thiamin) and Vitamin B6 (Pyridoxalphosphate), by automation with a Tecan Fluent and a Tecan LC-MS kit compared to the previously established lab-developed test with a Freedom Evo® automation. The focus was on evaluating improvements in sample tracking, processing efficiency, and resource consumption compared to conventional methods, and determining the suitability of this novel automated workflow for routine laboratory applications in vitamin status investigation.

METHODS.

Sample preparation was performed according to the Tecan LC-MS kit Ref # 30261534/30261535 (* and **). Whole blood samples were homogenized and extracted using a deproteinization solution. Isotopically labeled internal standards for each analyte were added (see Figure 1). Six calibrators and quality control samples at three concentration levels, within the expected physiological range, were reconstituted from a lyophilized whole blood matrix and processed as samples. The supernatant was injected into an LC-MS/MS system comprising a Thermo Scientific Vanquish UHPLC and a TSQ Quantis mass spectrometer operated by Chromeleon. For automated processing, a Tecan Fluent 780 liquid handler (see Figure 2) equipped with a robotic gripper arm and an 8-channel pipetting arm with piercing tips was used, along with a 4-slot plate centrifuge, four orbital shakers, and two tube rotators with integrated barcode scanners. The automated workflow is illustrated in Figure 3. Total durations for manual and automated sample preparation were recorded, and the analytical performance and environmental impact of both procedures were evaluated.

Figure 1: General workflow for analysing Vitamin B1/B6 by LC-MS.

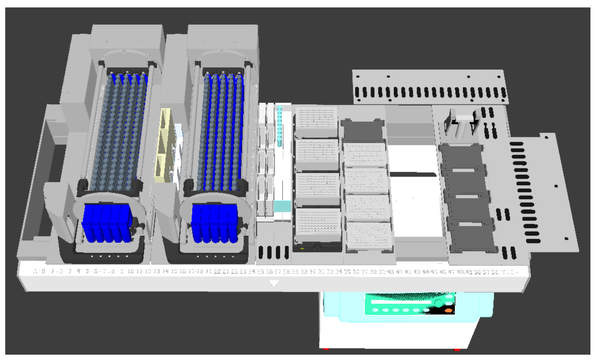

Figure 2: Illustration of the worktable of the Fluent 780 used within this study, highlighting two tube rotators, four Orbital Shaker and a centrifuge.

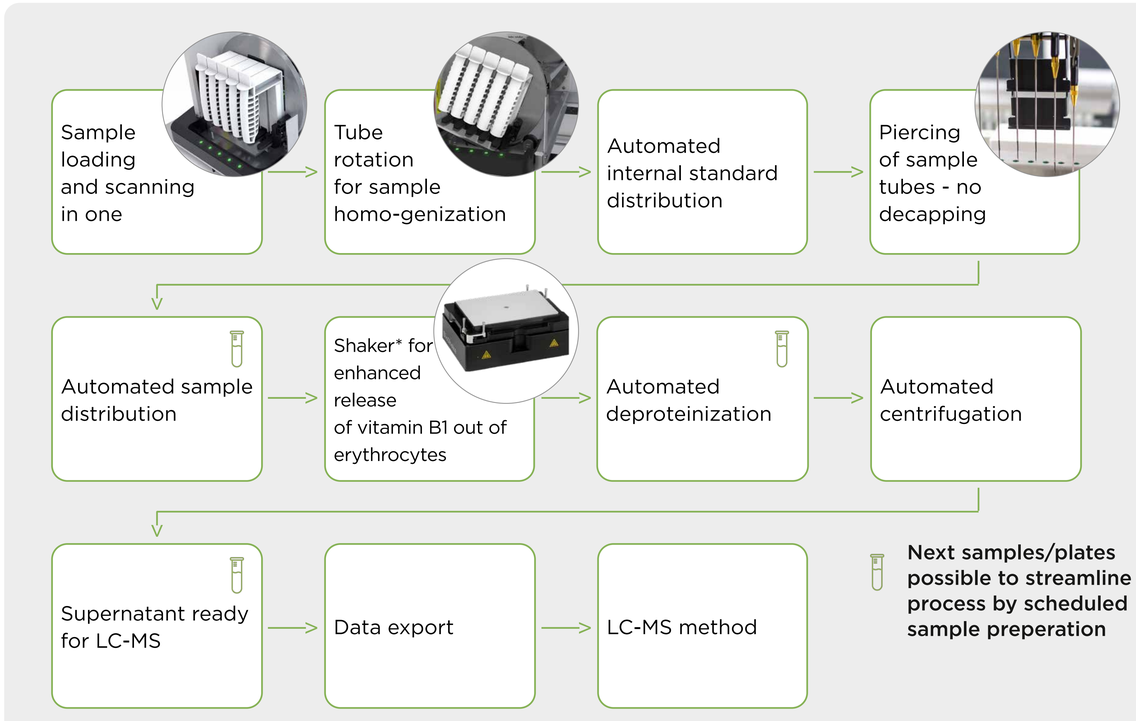

Figure 3: Workflow-illustration of the automated Fluent sample preparation used within this study.

RESULTS.

The average total duration per 96 well plate with the automated Fluent workflow in an eight-plate-scenario was approximately 41 minutes. The approximation included a sample scanning duration of 3 minutes, a plate transfer and shaking duration of 7 minutes, reagent distribution durations of 6 minutes, sample distribution durations of 15 minutes, centrifugation durations of 3 minutes and a supernatant transfer of 7 minutes.

The average total duration per plate with the established workflow in an eight-plate-scenario was 53 minutes. This included a sample scanning duration of 3 minutes, reagent distribution durations of 7 minutes, shaking and transfer times of 7 minutes, sample distribution times of 7 minutes, a centrifugation of 3 minutes a supernatant transfer of 7 minutes as well as a de- and recapping of sample tubes of 19 minutes. While this last step could be omitted within the Fluent workflow, pipette washing generally took longer than the uptake and discarding of disposable tips within the established workflow.

The analytical performance of both methods was comparable with calibration curves showing $R^2 > 0.99$ and control samples being in range. During the previous method, 208 pipette tips per plate were used and discarded as well as 192 sample caps for tubings. With the Fluent automation solely 96 pierceable sample caps were used while pipette tips were washable.

Table 1: Duration overview of manual versus Fluent sample preparation workflow.

Duration of Sample Preparation Steps	Manually	Fluent
Decapping and recapping sample tubes	19 min	n.a.
Tube-/Plate transfers	5 min	5 min
Sample scanning	3 min	3 min
Sample distribution	7 min	15 min
Reagent distribution	7 min	6 min
Agitation	2 min	2 min
Centrifugation	3 min	3 min
Supernatant transfer	7 min	7 min
Total	**53 min**	**41 min**

CONCLUSION.

The Tecan Fluent automation proved to be a robust and valuable enhancement of Vitamin B1/B6 LC-MS worklflows. Sample prep time and most importantly staff occupation has been shown to be reduced significantly compared to established workflow. The differences in pipetting durations were caused by the usage of either washable tips (Fluent) or disposable tips (previous method), however, the main impact on the overall workflow was the automated cap piercing. With its analytical performance and its significantly lower environmental impact, the novel automation workflow should be the preferred fit for a sustainable and state-of-the-art laboratory environment.

Disclaimer: The workflow presented here has not been validated by the manufacturer for use together as described.

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