SPOTLIGHT feature

LIMS & Lab Automation

Pushing the boundaries of automation in bioanalytical science: How far can we go?

Camilla Liscio, PhD, Element Materials Technology

Amongst the latest additions to the analytical technology portfolio, automation has become increasingly more established in the analytical workflow, especially after an unforeseen global pandemic, which tipped the balance for automation from a nice-to-have to a need-to-have tool.

Whilst automated sample introduction and instrumental analysis have been a constant in any laboratory for the past decades, the focus of automation has shifted towards the still unexplored aspects, such as automated sample handling or sample preparation. However, the development and optimisation of these steps are often far from trivial.

When implementing automated analytical workflows, the underlying framework relies upon translating the manual procedure to its automated analogue. To do that purposefully, it is crucial to understand the curses and the needs of the relevant community.

In bioanalytical science, processes are often cumbersome and time-consuming; human error can occur unpredictably, combined with a heavy operational workload. Nevertheless, data quality remains of utter importance, and there are scenarios where sample throughput and turnaround time are crucial drivers.

This article shares three case studies exploring different tactics for automating bioanalytical science workflows. It will highlight the advantages and limitations of the automated approach and showcase some of the latest developments, giving access to additional automation capabilities.

Insights on analytical automation technology

Analytical automation has evolved over time, and its evolution has been strongly connected to the technology developments. Robotic platforms started as autosamplers to automate sample introduction and improve sample analysis. Those limited liquid handling capabilities have been further implemented to create automated liquid handlers for serial dilution. The next logical step was to move into automated 'preppers'.

Preppers exist in various formats, depending on the tasks and the throughput the users require. The most established options fall within the well-plate and vial formats. The well-plate format features robotic arms, automated liquid handlers, and dedicated prep stations. In contrast, the vial format uses instrument top X-Y-Z robotic platforms fitted with various prep modules. These two formats are somehow complementary and show opposite strengths and weaknesses; hence, it is fundamental to identify the critical workflow drivers to choose the most relevant automation option.

The three following case studies showcase the versatility and customisation of the vial format, which lends itself to the automation of complex sample preparation.

Case study 1: Translating manual to automated

This study was done in collaboration with Dr Rachel Carling, Director of Newborn Screening and Clinical Lead, Biochemical Sciences at Synnovis, the pathology provider to Guys & St Thomas' NHS Foundation Trust, London. It focused on translating their existing manual method for the analysis of organic acids in urine to a fully automated solution. Analysis of organic acids is a powerful approach to diagnosing inborn metabolic disorders. The most well-established method is a GC-MS screening, which requires extensive preparation, including an oximation step, liquid-liquid extraction, an evaporation step, and a final silylation step before injection. The entire workflow was automated using a GERSTEL Dual Head Robotic Multipurpose Sampler (MPS) fitted on top of an Agilent GC-MSD. Online automated solutions are highly advantageous as they allow staggering the sample preparation and GC runtime along the batch, optimising time and minimising time degradation issues.

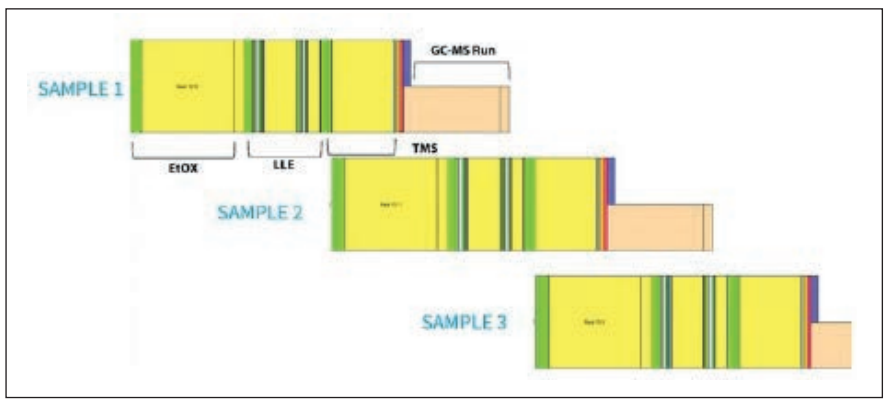

Figure 1: Timelines for the online extraction, derivatisation and GC-MS analysis of urine samples using online automation.

In fact, TMS derivatives have limited stability and the prompt injection after sample preparation in this case permitted reduced variability over randomised batches. Furthermore, automation allowed fine-tuning control of temperature, time, and pressure during the evaporation step, which guaranteed the retention of the volatile short-chain organic acids, some of which might be diagnostic. A custom library for the ethoxylated TMS derivatives was built using relevant IQC material. Quantitative data for two target acids, methylmalonic acid and orotic acid, were compared for the manual and the automated methods. Linearity was comparable for the two approaches, and Bland–Altman plots revealed satisfactory agreement between the manual and automated methods. Thirty-seven patient samples were screened using the in-house custom library, and 35 organic acids were specifically checked for detection. Only 14 chromatograms did not fully align. Responses for the diagnostic organic acids across the analysed samples were successfully confirmed for both the manual and automated methods.

Case study 2: Reinventing the current approach

This project was done in collaboration with Dr Eylan Yutuc and Professor William J. Griffiths at Swansea University Medical School. It challenged the standard manual-to-automated translation framework as it required reinventing aspects of the existing approach. In lipidomics, oxysterols and sterol- acids in human plasma are valuable markers to diagnose inborn errors of metabolism and monitor neurodegeneration. However, sterols' solubility in highly aqueous mixtures is limited, even when derivatised. This can impact the performances of the commonly used reverse-phase solid-phase extraction (RP-SPE), leading to a lack of retention and analyte losses. To circumvent the problem, a recycling approach can be implemented, where the loading is collected, adjusted gradually in polarity and reloaded several times before committing to the final elution [1]. Automating this step offers the advantage of delegating a very repetitive task, easily prone to error, whilst improving the robustness and transferability of the method across several laboratories. The standard automated SmartSPE® module was customised by introducing a 3D-printed collection station for the large recycling volumes *(Figure 2)*. A scale-down factor was applied to the whole procedure to accommodate both the smaller bed size featured in the miniaturised cartridges and the robotic platform optimal liquid handling capabilities.

A selection of sterols standards ranging in polarities and functionalities was tested to assess the automated method's precision and recovery performance. Percent Relative Standard Deviations (n=3) ranged between 2% and 17%, whilst recovery was between 83% and 112%. A direct comparison of the manual approach with automation was carried out using plasma QC samples. Overall, a good agreement between the two approaches was observed across the whole range of target analytes, and the scale-down factor did not impact the method's sensitivity.

Case study 3: Thinking outside the box

This work resulted from a multidisciplinary collaboration with Neoteryx- brand of Trajan Scientific, ILT, and Dr Rachel Carling's team. It focused on developing an innovative approach to automate the handling of blood microsampling devices. Over the past decade, microsampling has gained extensive attention in laboratories as it allows blood collection from a finger prick rather than venipuncture blood sampling.

Figure 2: Customised recycling station for the automated miniaturised SPE of derivatised sterols in plasma

The low volume of biological matrices used in microsampling has made analysis less invasive, facilitated testing implementation and lowered costs. Based on a patented VAMS® (Volumetric Absorptive Micro Sampling) technology delivered delivered via porous, hydrophilic polymeric tips mounted onto plastic sampler bodies similar to pipette tips, Mitra® devices are designed for remote collection of a fixed blood volume. However, manual removal and transfer of the Mitra® tip from the collection device into the extraction vessel is often prone to human error, potentially raising patient safety and quality concerns. Automated VAMS® technology not only reduces these risks by delegating the transfer task to the robotic platform, but it also offers additional benefits in terms of transferability across laboratories and sample traceability. To automate the handling of Mitra® devices, three new hardware capabilities were implemented on the robotic platform: Mitra® storage using Mitra 96- Autoracks®, Mitra® tool to transport the sampler bodies, and Mitra® station for the Mitra transfer to hold in place the vial whilst the Mitra® tip was pushed through the pre-slit cap into the vial *(Figure 3)*.

The optimised hardware configuration was tested on a real case scenario by comparing the manual versus automated approaches to analyse the immunosuppressant tacrolimus in the blood of 284 kidney transplant patients [2]. Passing Bablock regression was used to determine whether there was a correlation between the two methods.

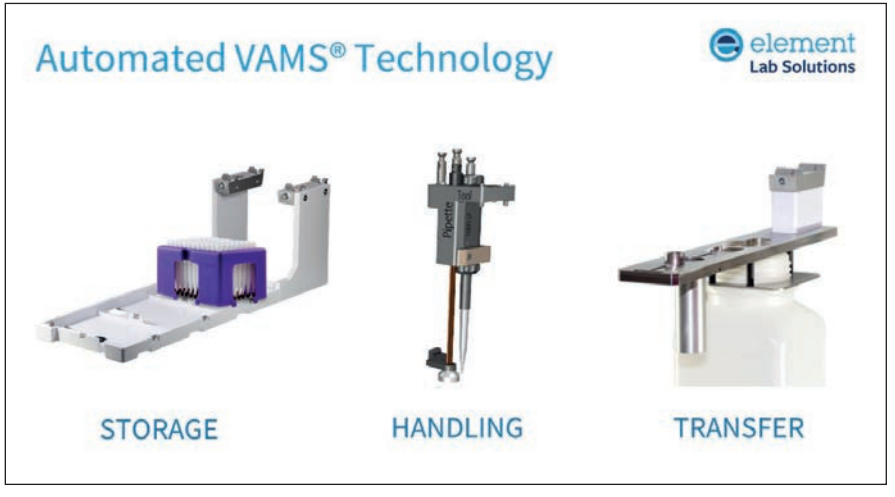

Figure 3: Automated VAMS technology hardware.

The regression showed a proportional positive bias of 6% for the automation and a constant positive bias of 0.45 ug/L, both considered acceptable. Bland-Altman plot showed satisfactory agreement between the methods and highlighted a minor positive bias for the automation of 0.007 ug/L.

Conclusions

These three case studies showcased the potential and limitations of vial format automation in bioanalytical science applications. Speed and throughput are often challenging to meet because of the single-channelled embedded approach; fine-tuned development is crucial to address the workflow complexity, and scale-down requirements can potentially impact the overall sensitivity of the method. However, very complex workflows, heavily affected by human error, were automated from start to finish, and with the example of automated VAMS technology, analytical automation has been proven capable of including sample handling in the whole automated process.

References

1. Free Radical Biology and Medicine, 59 (2013) 69-84
2. Bioanalysis 14 (2022)1487-1496