Automation meets precision analytics: A paradigm shift in HPLC throughput methodology

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Introduction

Developing an efficient microbial strain is an inevitable cycle in the precision fermentation industry, as it translates directly into product quality, stability and cost. Biological systems are inherently complex due to their multiple metabolic pathways and genetic regulatory mechanisms. Hence, developing an optimal strain would demand multiple distinct engineering strategies to be combined to generate a comprehensive strain library. Identifying high-performing candidates from these libraries is often labor-intensive, as it involves screening large numbers of microbial colonies. Thus, an effective high-throughput analytical tool is an essential part of the strain development process that can significantly reduce sample processing time, manual intervention and, thereby, the error rate, and improves the reproducibility of the results.

Perfect Day is building a sustainable and resilient food system by harnessing the power of precision fermentation to make heterologous commodity proteins at a commercial scale. They use various transcriptional and translational toolboxes to engineer strains, thereby rapidly evaluating thousands of clones that show superior product traits.

In the current work, we describe how we unlocked the potential of using a high-throughput Biomek i7 liquid handler to increase reproducibility and to reduce sample preparation time during evaluation of hundreds of clones in high-performance liquid chromatography (HPLC). The method involves preparation of a deep well plate with culture supernatant samples harvested from fungal cultures directly into the HPLC glass vials ready to go for HPLC injection. By integrating the Biomek i7 workstation with the Perfect Day strain engineering workflow and analytical pipelines, we demonstrate a reduction in overall sample processing time while generating accurate, reproducible HPLC results.

Spotlight

The workflow involves sample preparation of engineered strains. Samples are harvested from DWPs (both 24- and 96-well formats) to 96-well plate using an automated protocol developed in-house using the Biomek i7 liquid handler. Part of the Biomek i-Series platform, the i7 workstation operates on air or liquid displacement technology to ensure precise and consistent sample handling via both multichannel and Span-8 pipetting heads. In the current workflow we explored the Span-8 head in a Pod 2 module comprising eight independently controlled probes. As shown in Figure 1, the liquid handling system (LHS) deck is configured for HPLC sample preparation following sample harvest.



Figure 1. Deck layout of Biomek i7 liquid handler with the required consumables for performing sample preparation in the HPLC glass vials.

Following automated sample preparation, samples are subjected to high-performance liquid chromatography (HPLC) for quantification of the protein of interest (POI). HPLC data is then analyzed to identify top-performing clones, which are selected for further validation and scale-up studies in shake flasks. This integrated automation workflow significantly streamlines high-throughput screening and accelerates hit selection.

Experimental setup & methods

The culture supernatant harvested in 96-well microtiter plates (MTPs) is used for the HPLC analysis to determine the concentration of POI. Based on standardized dilution ranges for different types of strains/clones, the dilution of samples in the HPLC vials was performed. HPLC vials (Type I Borosilicate Glass-printed screw vial clear 2 mL, diameter of 9 mm) were mounted on a 12 mm insert tube on a 24-position tube rack holder (both from Beckman Coulter Life Sciences). Biomek i-Series 1070 µL pipette tips and sterile 230 µL pipette tips are used for diluent and samples, respectively. Modular reservoir (quarter module - 40 mL from Beckman Coulter Life Sciences), along with the reservoir frame was used for dispensing HPLC sample diluent as shown in the layout of the Biomek system shown in Figure 1. The workflow of automated sample preparation, beginning from mapping the Biomek system deck to sample preparation, is shown in Figure 2.

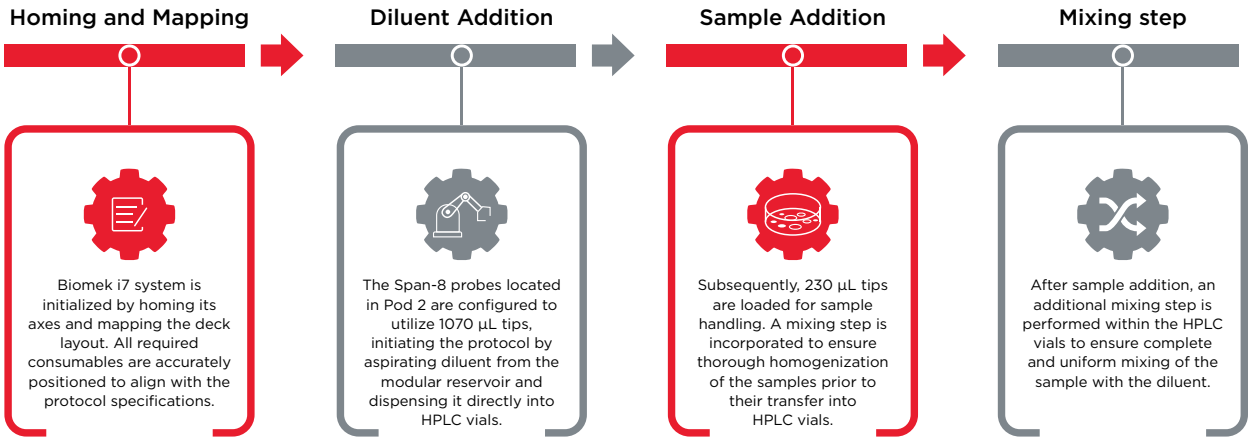


Figure 2. Established workflow for the automated protocol implemented on the Biomek i7 liquid handler, enabling direct sample preparation into HPLC vials, which are subsequently positioned in the autosampler for injection into the HPLC system.

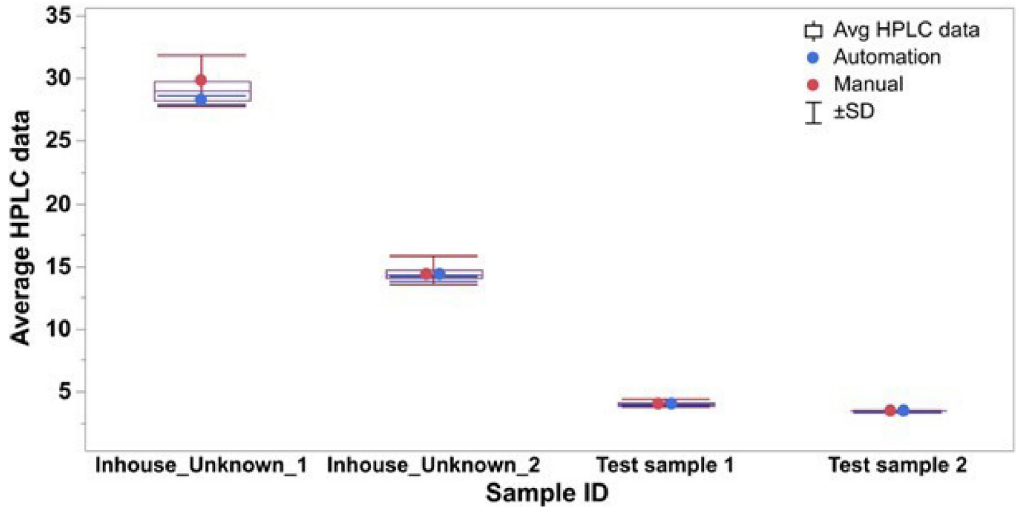
The sample preparation volume is standardized for a final volume of 500 µL, with the protocol adjustable for initial and final volumes of preparation. Required diluent volume is aspirated from a modular reservoir and dispensed directly into HPLC vials using 1070 µL tips, as illustrated in Figure 1. Subsequently, samples are mixed in a 96-well plate, followed by aspiration of required sample volume and transfer into HPLC vials. Final mixing is performed using 230 µL tips to ensure homogeneity.
The protocol integrates adjustable parameters including mixing volume and speed, aspiration and dispensing heights—optimized based on sample-specific attributes such as viscosity, media composition, and consumable type. These settings are adaptable to varying experimental conditions.

Results

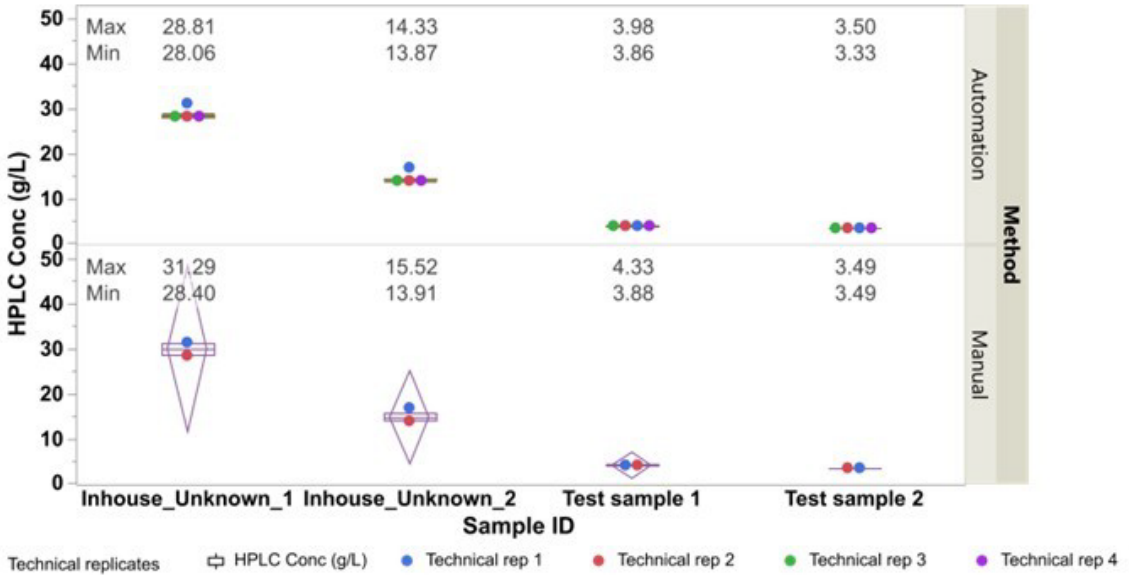
1. Highlights of the automated protocol

To compare and validate the performance of the automated protocol with manual workflow, technical replicates were generated for both the methods. Using the Biomek i7 liquid handler, four replicates were prepared for each of the samples, comprising two unknown samples and two DWP test samples, while manual preparation involved two replicates per sample. All preparations maintained consistent dilution ratios, diluent volumes, and sample volumes to ensure comparability.

(A)



(B)



(C)

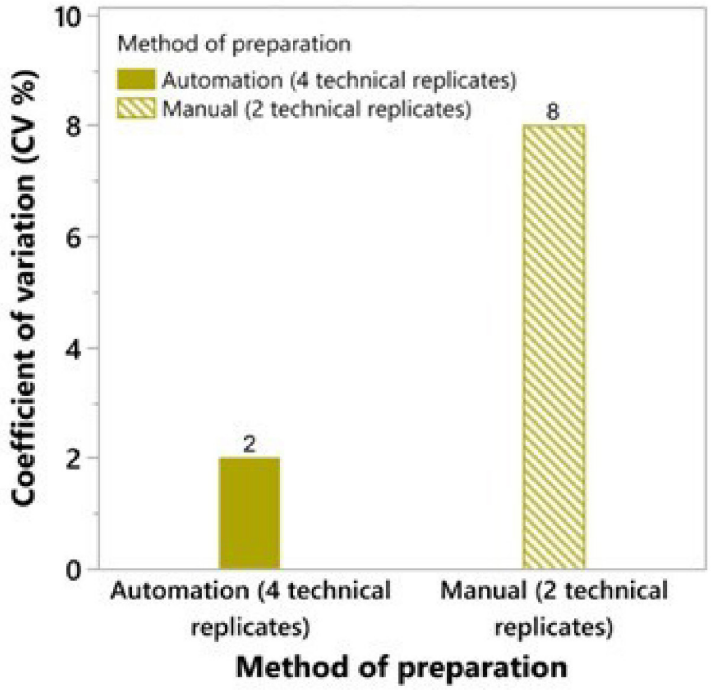


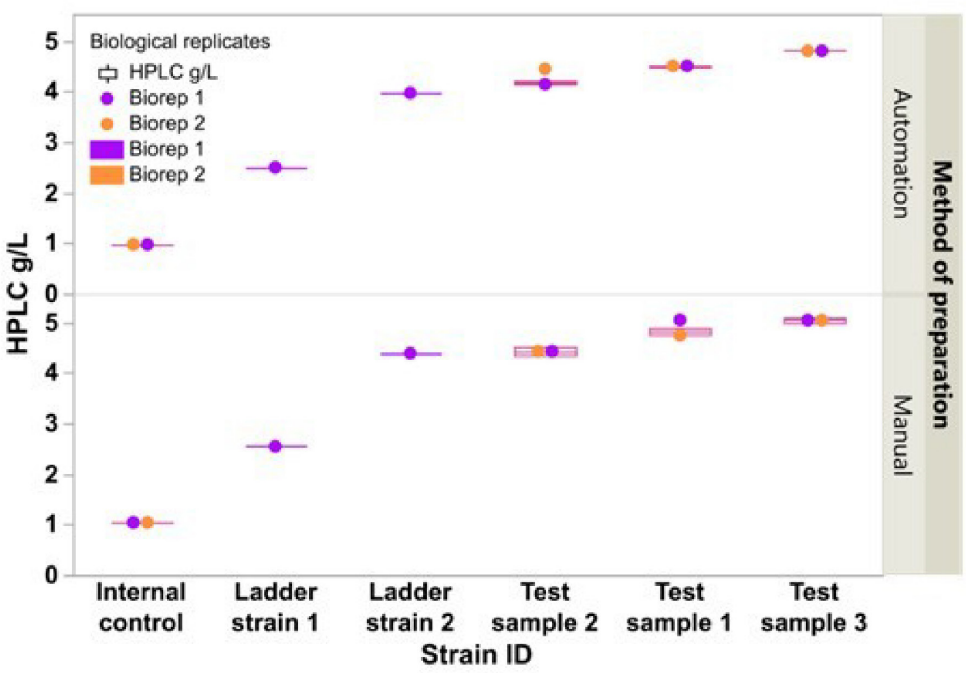
Figure 3. (A) Standard deviation of average HPLC (g/L) results for both preparation methods, indicating statistical variation. (B) Distribution of data points from HPLC method for in-house unknown and test samples prepared via manual and automated workflows, illustrating data tightness. (C) % CV between manual and automated workflows, reflecting differences in reproducibility.

Perfect Day's optimized and validated automated protocol is observed to provide higher statistical confidence even with large sample size, as illustrated in Figure 3 (A) and (B), where the standard deviation bar is smaller for automated preparation. This data in turn highlights that in-house automated protocol not only improves throughput and efficiency of sample preparation but also reduces error rate and enhances data accuracy. Technical replicates prepared by automation exhibited a % CV of ~2, while manual preparation exhibited CV of 8%. With more technical replicates exhibiting much less variation, automated platform enhances reproducibility for unknown and test samples.

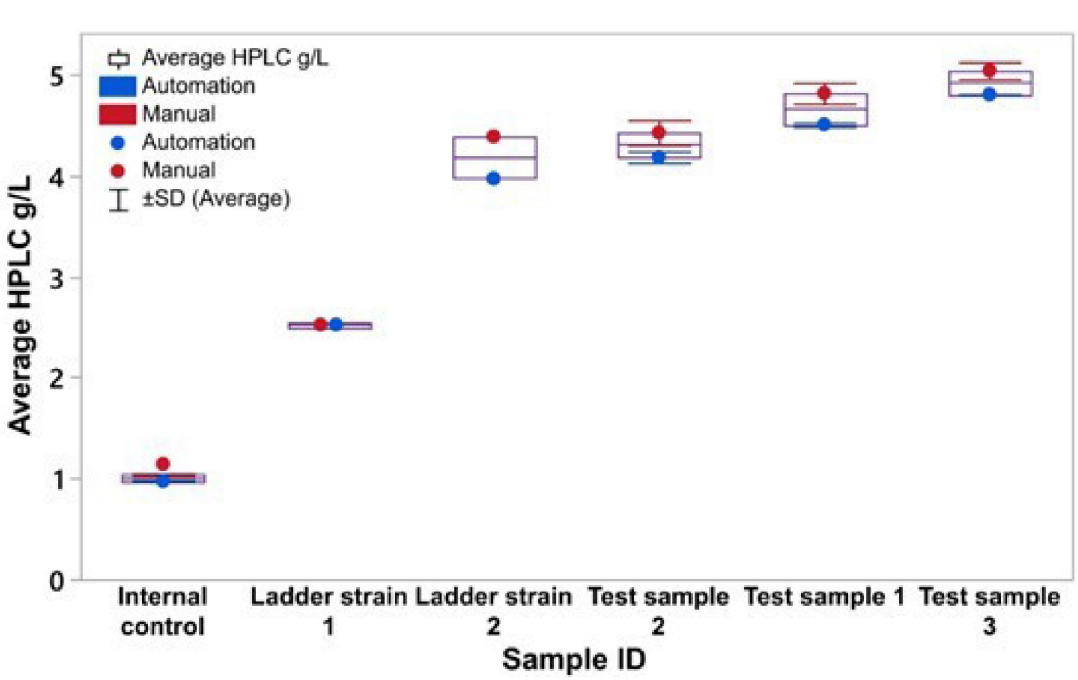
2. Supporting streamlined analysis

To assess the robustness of the protocol under routine conditions, the performance of the protocol was evaluated using biological replicates along with internal controls and ladder strains. Ladder strains are benchmark strains used in every experiment as a control. The samples analyzed in this study exhibited high complexity, primarily due to high levels of host cell proteins (HCPs) present in the background matrix.

(A)



(B)



(C)

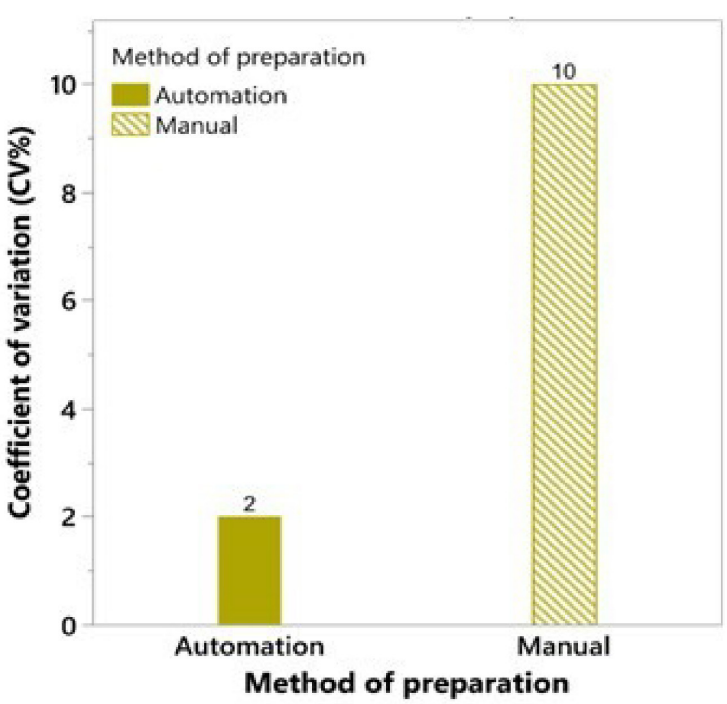


Figure 4. (A) Consistency of biological replicates for Ladder strains that are benchmark strains of varying concentrations of POI and test samples prepared via manual and automated workflows. (B) Standard deviation (g/L) of average HPLC measurements within biological replicates for both sample types, reflecting statistical variation across preparation methods. (C) Biological replicates under manual and automated workflows.

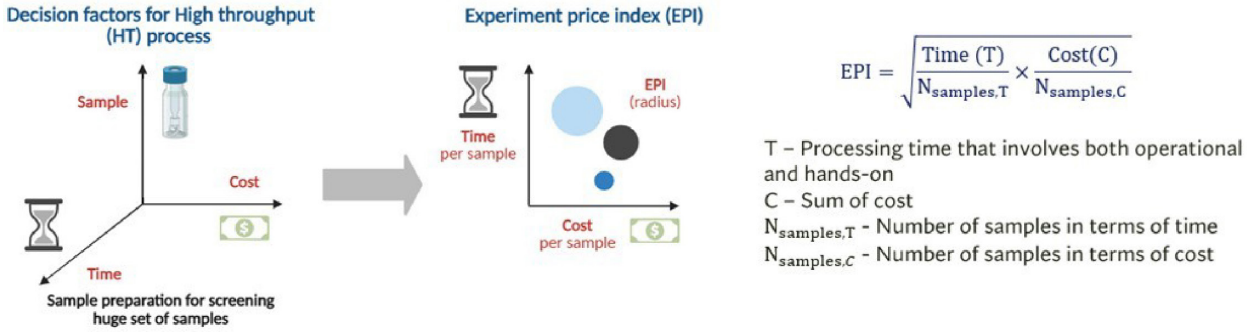
As illustrated in Figure 4 (A) and (B), biological replicates prepared using the automated workflow exhibited lower variability and more consistency between biological replicates compared to those processed manually. While high levels of HCPs can interfere with analysis such as HPLC, reduced variability with automation suggests that the automated workflows produce robust data to select true top hits in high-throughput screening regardless of the matrix. For biological replicates, observed CV% is ~2 for automated process, while ~10% CV was observed in manual preparation. The increased variation observed in manually prepared samples may be attributed to inconsistencies involved in manual handling, which are often beyond the control of the individual performing the preparation. This in-house validated automated workflow generates biologically robust replicates with reduced inter-sample variability, indicating strong analytical precision for this method.

3. Cost efficiency

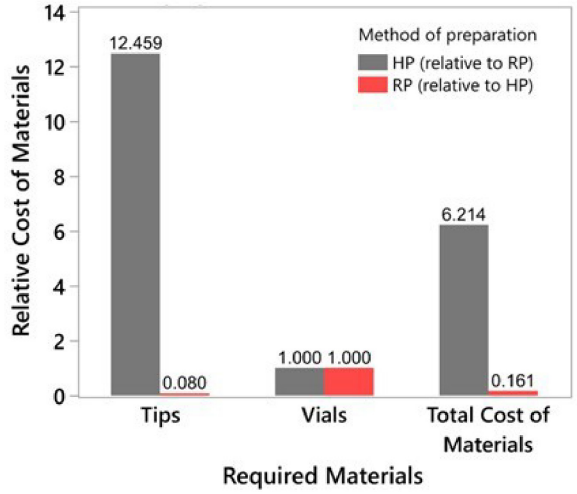
To evaluate the economic impact and efficiency of laboratory automation using robot-assisted workflows, the Experiment Price Index (EPI) is employed as a primary quantitative metric. EPI enables comparative assessment across key parameters such as time, cost and sample throughput. This uses geometric mean-based calculation to standardize per-sample expenditure relative to specific experimental objectives [1]. Thus, targeting lower Experiment Price Index (EPI) values enables identification of cost-efficient protocols, thereby enhancing overall experimental throughput and resource utilization.

The efficiency of a robotics-assisted process (RP) versus a human-powered process (HP) for samples from 8 96-well microtiter plates over a period of one month (~22 working days) was assessed. Cost per sample preparation was estimated (including consumables and reagents required in the process) for both the means (RP and HP). Cost per sample preparation was observed to be lower in RP than HP, due to use of a tip washer installed in the Biomek i7 liquid handler, while consumables such as tips cannot be reused during HP. For comparison, relative cost of materials (C_M) for HP and RP for sample preparation in HPLC vials was estimated as shown in Figure 5. C_M for HP is observed to be 6.214-fold higher than C_M for RP.

(A)



(B)



(C)

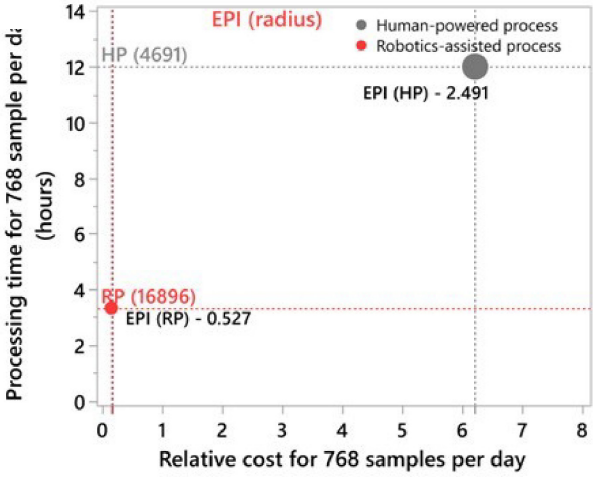


Figure 5. The experiment price index (EPI) to economically evaluate sample preparation workflows. (A) Representation of process decision factors (cost, time and number of samples) for HT experiments along with the formula for EPI calculation [1]. (B) Detailed relative cost for HP and RP sample preparation in HPLC vials for a period of 1 month, where samples filled in 8 MTP were processed in a day (~768 samples/day, where RP can process ~16,896 samples over a period of 1 month, while HP can process ~4,691 samples, provided the individual takes short breaks throughout the manual workflow). (C) Bubble plot of EPI calculated for HP and RP for same period of time.

Note: EPI calculation assesses operational expenses (OpEx) intrinsic to the experimental workflow and excludes capital expenditures (CapEx) associated with installation or ongoing maintenance expenditures.

EPI for both HP and RP was estimated using the formula shown in Figure 4 (A). EPI for RP (~16,896 samples) was calculated to be 0.527, while for HP (~4,691 samples) the EPI was observed to be 2.491. It is evident that RP exhibits low EPI, which is identified as a more cost-effective procedure improving overall experimental efficiency. The number of samples for both methods indicates the number of samples that can be prepared in a particular duration (~3 hrs/day for 22 working days a month). The throughput numbers are calculated based on the number of samples prepared for a week. This was then extended for a duration of one month, where 22 working days were considered. In addition, EPI for HP was calculated to be 4.72-fold higher than RP. This in turn demonstrates how efficient the process is when a Biomek i7 liquid handler is integrated in the experimental workflow, where automation is more efficient in terms of time, cost, and number of samples prepared with more accuracy.

Discussion

Laboratory automation has become a cornerstone of modern biotechnology, offering substantial improvements in throughput, reproducibility and overall workflow efficiency. Integration of the Biomek i7 liquid handler into a workflow for the HPLC sample preparation process has significantly enhanced both the consistency and scalability of experimental data. The developed protocol has enabled the screening of a larger number of strains without the need of highly laborious and error-prone manual sample preparation. The automated platform enables parallel processing of samples, precise pipetting and controlled mixing, significantly reducing preparation time while enhancing consistency. Additionally, the incorporation of auxiliary components such as barcode scanners, sample tracking systems, and automated storage units further extends the platform's capabilities, supporting scalability and traceability across similar analytical pipelines.

Additionally, automation-enabled sample preparation was made cost-efficient by incorporating a tip-washer. The use of the Biomek i7 liquid handler contributed to a notable 83.9% reduction in sample preparation costs. These findings underscore the transformative impact of automation: enabling Perfect Day's platform to achieve higher throughput, greater cost-efficiency, improved accuracy and more robust, reproducible data.

Perspectives

Within the broader bio-separations landscape, effective sample preparation is essential for bridging complex biological matrices with analytical platforms such as mass spectrometry and HPLC. Integrating liquid handling platforms such as the Biomek i7 workstation with HPLC workflow enables semi- or fully automated, high-throughput, and reproducible sample preparation workflows for diverse applications. Integration of the liquid handling system not only enhances reproducibility, throughput, and scalability but also can be cost- and time-efficient.

References

1. H. M. &. K. J. Woo, "Measuring the economic efficiency of laboratory automation in biotechnology," Trends in Biotechnology, pp. 1-5, 2024.
2. Automating the Valita Aggregation Pure Assay on a Biomek i-Series Liquid Handler
3. Application Notes, Browse Scientific Resources

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This application is not intended or validated for use in the diagnosis of disease or other conditions.

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