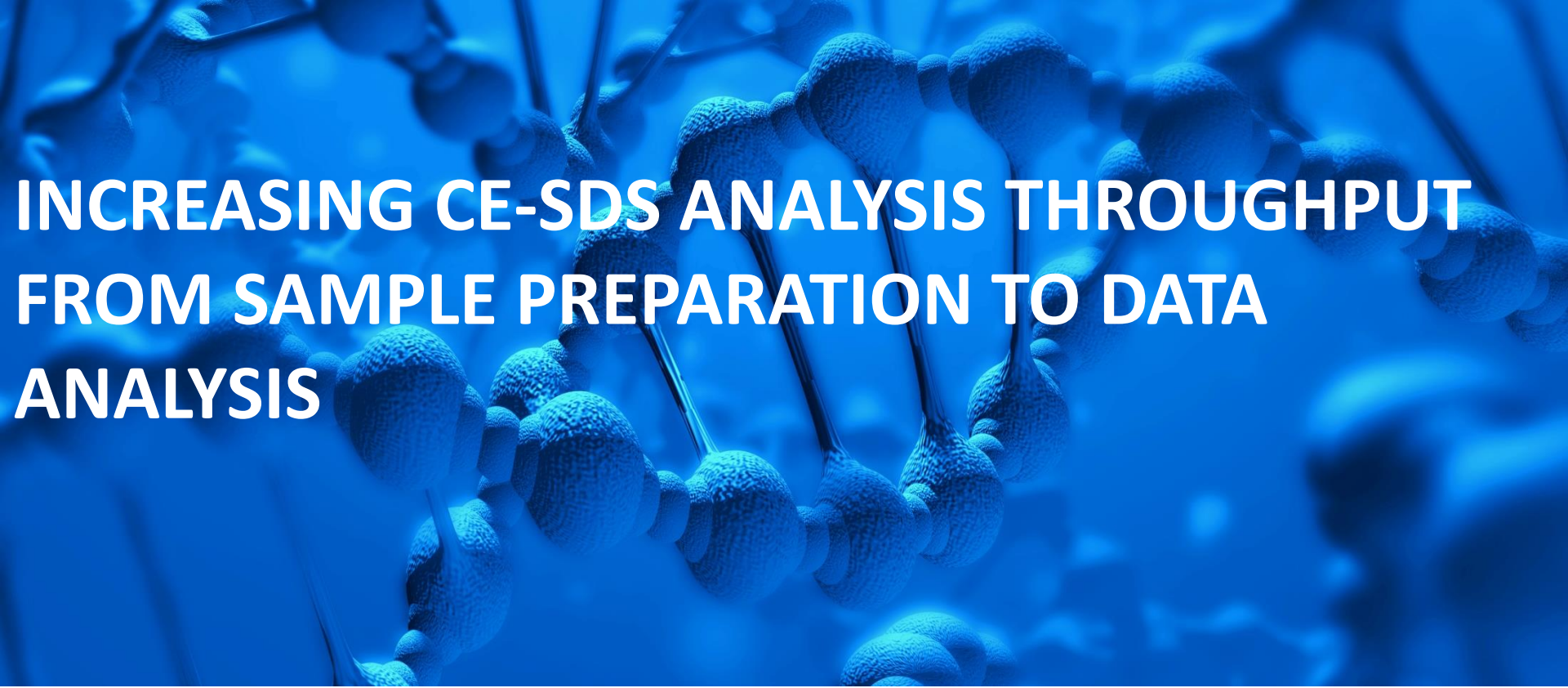

INCREASING CE-SDS ANALYSIS THROUGHPUT FROM SAMPLE PREPARATION TO DATA ANALYSIS

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ABSTRACT

Capillary Electrophoresis-Sodium Dodecyl Sulfate (CE-SDS) is a separation technology widely used in the BioPharma industry to assess product purity based on differences in the hydrodynamic size of clipped, intact and aggregated species under denaturing conditions. Sample preparations for CE-SDS can be cumbersome, time-consuming, and involve many pipetting steps. Manually setting up one run requires approximately up to two hours of hands-on analyst time.

To automate a complex sample dilution and preparation procedure, two liquid handlers have been evaluated. The first automated sample preparation includes the completed script from sample concentration normalization, multiple reagent additions, mixing to ensure homogeneity, incubation, and final transfer to its ready-to-analyze state. The sample normalization volumes are fed directly to the automated prep #1 script via a stand alone web based application, thus reducing analyst set-up time and dilution errors. The automated prep #1 script encompasses the entire process, is easy to use, and reduces the risk of error. Because the final sample plate is in 96-well format, this automated process has increased operational efficiency and overall process capacity.

Data analyses can be done using a different software that can also be automated with the set criteria in each of the processing method. Each CE instrument has its own Chromatography Data System (CDS) that can be modified per user request. Automated data processing can be achieved by customizing the integration parameters applicable to each sample set, thereby reducing analyst time with manual data analysis. However, individual automated sample integration will still need to be examined to ensure correct peak identification and to provide the best data quality.

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OUTLINE

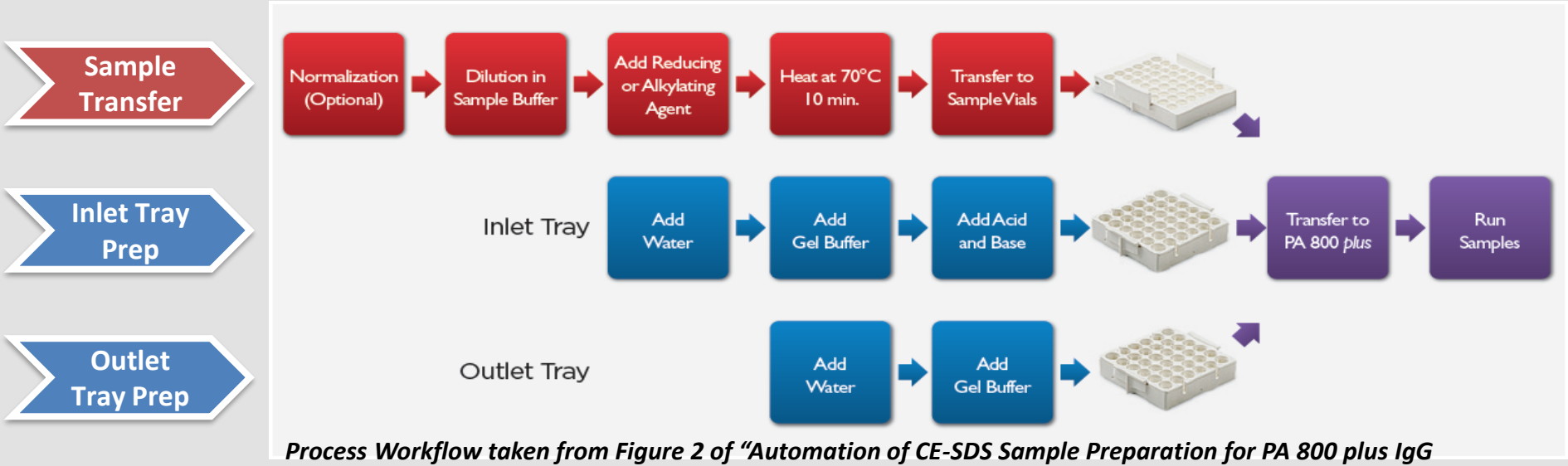
- Automation of CE-SDS Sample Preparations
- CE-SDS Data Analyses Tools Available
- Future Work and Conclusion

MANUAL VS AUTOMATED CE-SDS SAMPLE PREPARATIONS

Considerations	Manual Prep	Automated Prep #1 (Biomek 4000 Prep)	Automated Prep #2 (Microlab Prep)
Footprint	N/A	122.5 cm (W) x 50.5 cm (D) x 67.5 cm (H)	53.4 cm (W) x 61.0 cm (D) x 61.0 cm (H)
Ventilation Requirement	Standard Fume Hood	Special Ventilation Installation	Fits in Standard Fume Hood
Cost	N/A	~$55K (+$20K Ventilation Cost)	~$30K
Hands-on Time per Batch (Hour)	1.5	0.7	TBD
Maximum Number of Batches per Day	4	5	TBD
FTE Savings per Year	N/A	0.3 FTE/Year	TBD/year

MANUAL SAMPLE PREPARATION AND CHALLENGES

CE – Capillary Electrophoresis, is a separation technology, widely used in the BioPharma industry for Protein analysis to confirm protein purity and detect protein impurity levels.



Process Workflow taken from Figure 2 of "Automation of CE-SDS Sample Preparation for PA 800 plus IgG Purity/Heterogeneity Assays Using a Biomek 4000 Automation Workstation"

Challenges: vial format, repetitive pipetting, exposure to hazardous reducing or non-alkylating reagents (safety concerns with reducing agent, beta-mercaptoethanol if used for rCE-SDS)

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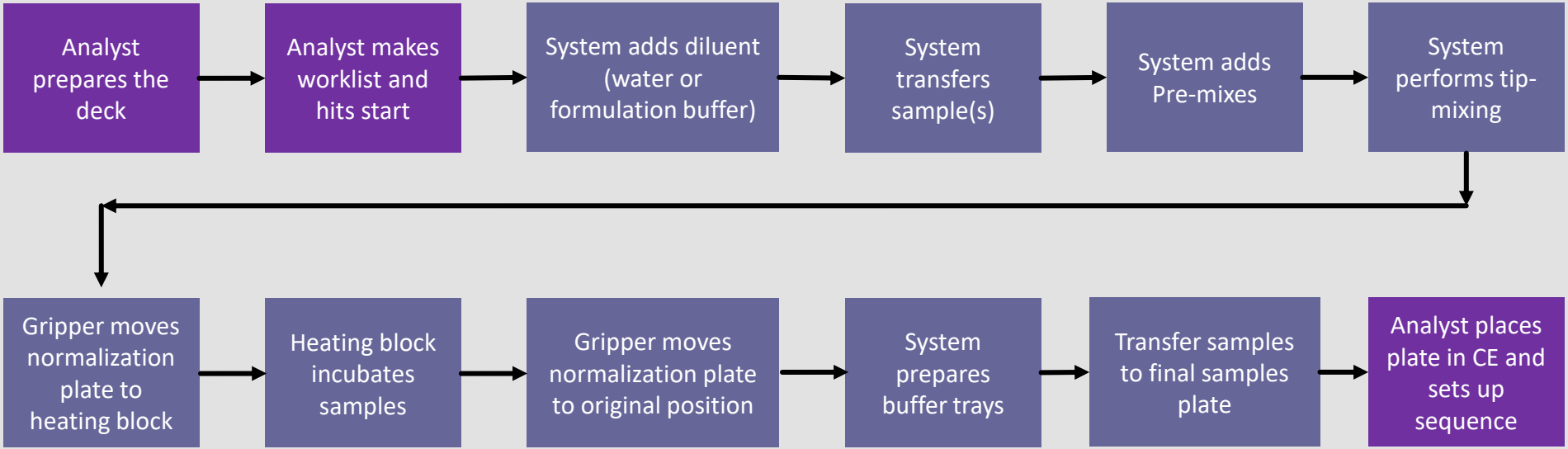
AUTOMATED PREP #1 SAMPLE PREPARATION STEPS



Analyst Time: 0.7 hours

Automated Prep #1: Max 1.5 hours for 19 samples

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AUTOMATED PREP #2 CE-SDS WORKFLOW

A. Dilute samples

Sample

Reducing reagent for reduced CE-SDS (rCE)

Sample Buffer

OR

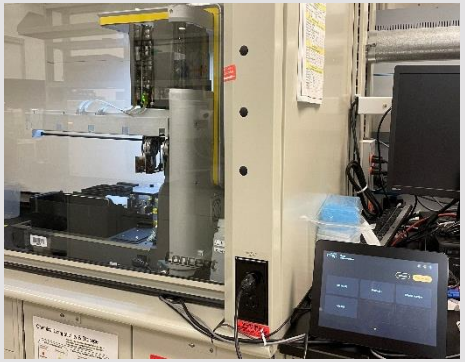
Non-alkylating reagent for Non-reduced CE-SDS (NR CE)

B. Prepare NR or rCE Premix

Normalized samples

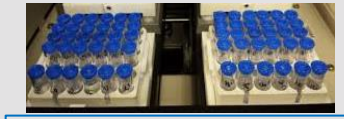
NR or rCE Premix

C. Transfer Premix to samples, and incubates plate



Treated samples

D. Prepare buffer trays



E. NR or rCE-SDS analysis

A & B: Manual; **C: Already automated**; **D: Can be automated**

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DATA PROCESSING TOOLS

Software	Manufacturer	Specific Feature	Unique Challenge
32 Karat	Sciex	Built into PA 800 plus	Report template needs to be customized per user
Compass	ProteinSimple	Built into Maurice	
Chromeleon	Thermo Fisher	Excel-based; Able to analyze data from 32 Karat and Compass	Sequences can be unlocked by other users
Empower	Waters	Report function has more features compared to 32 Karat and Compass; Utilize for both run acquisition and data analysis with full audit trail; Able to analyze data from 32 Karat, Compass and Chromeleon	Slow when processing large data files or sample sets greater than 12 injections

CURRENT RUN AND DATA PROCESSING WORKFLOW

PA 800 Plus run controlled by 32 Karat or Empower

Run samples

Maurice run controlled by Compass

Quick visual review of electropherograms

1. **If run on 32 Karat control, then export and import into Chromeleon or Empower**
2. **If run on Empower control, then process on Empower**

If run on Compass, then export and import into Chromeleon

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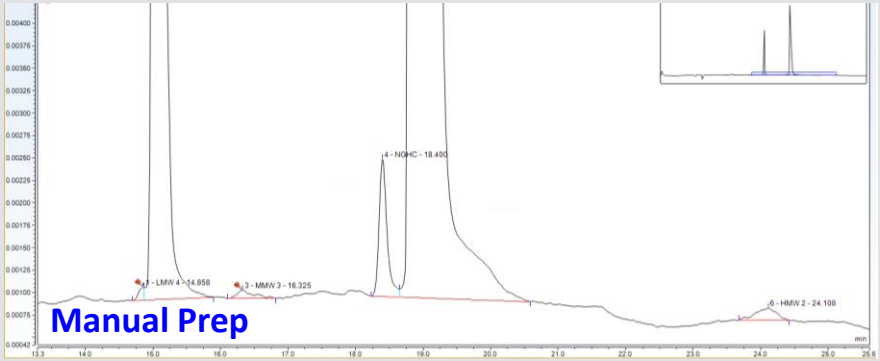
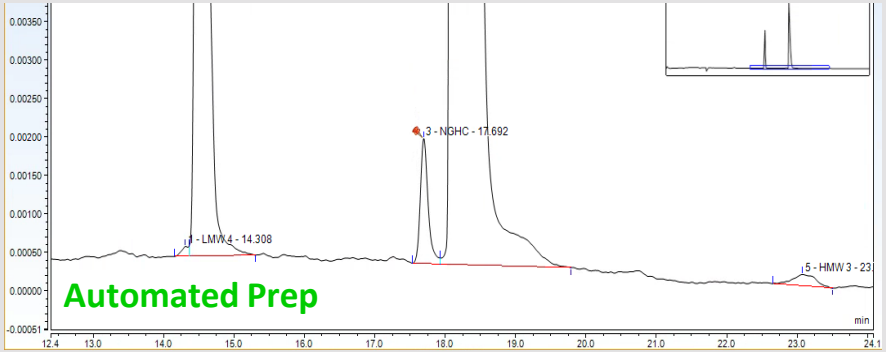
PROTEINSIMPLE MAURICE COMPASS SOFTWARE

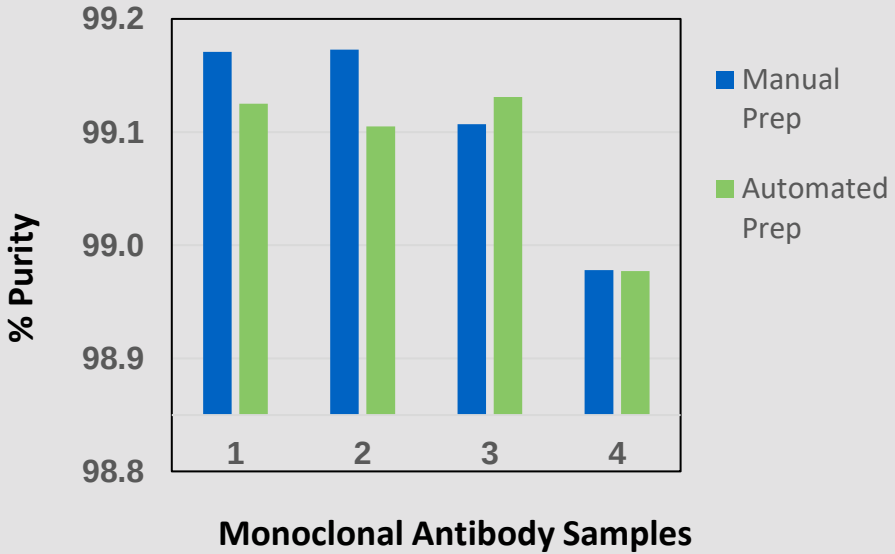
- New data smoothing feature now in SDS plus program
- Report template needed enhancement:
  - Modify default report template
    - Group multiple injections available with mean, standard deviation, and % relative standard deviation
  - Add baseline noise feature to understand what is calculated for limit of detection for minor peaks
  - Align decimal places in recording/reporting requirement
  - Add expanded electropherogram into report template
    - Full and zoom views

MANUAL VS. AUTOMATED PREPS REDUCED CE-SDS DATA
MONOCLONAL ANTIBODY (MAB) #1

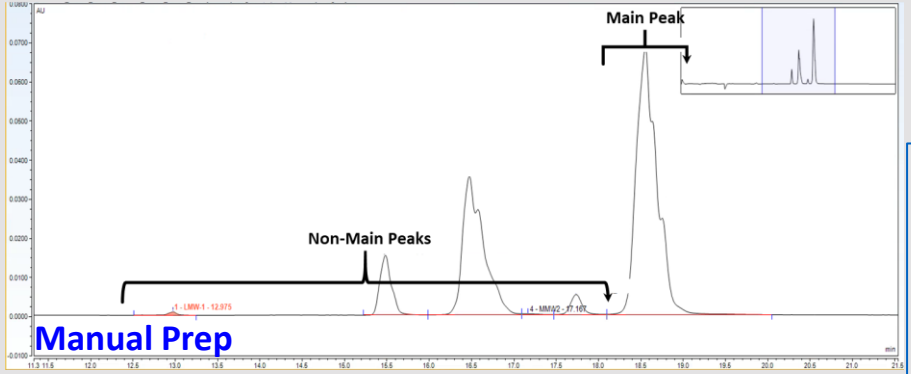




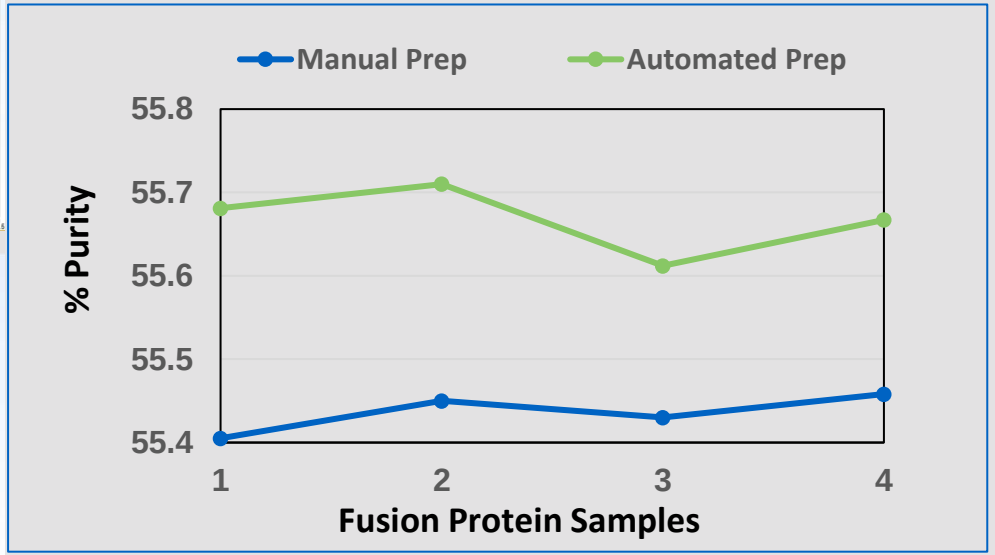
Sample profiles are comparable, with less than 0.07% absolute difference in % Purity.



MANUAL VS. AUTOMATED PREPS REDUCED CE-SDS DATA FUSION PROTEIN

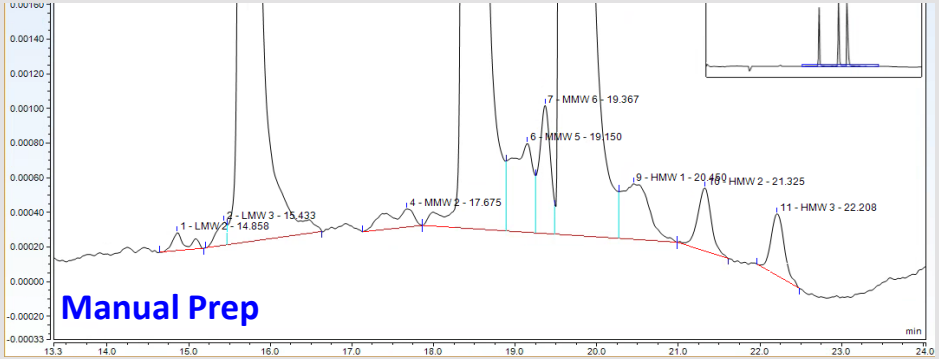


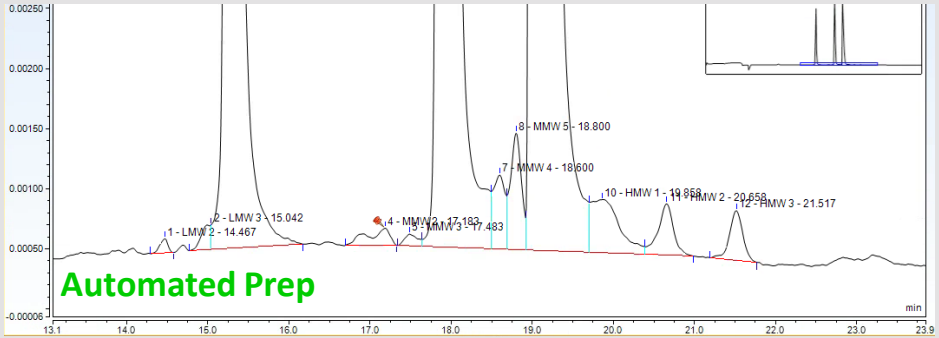
Sample profiles are comparable, with less than 0.5% absolute difference in % Purity.



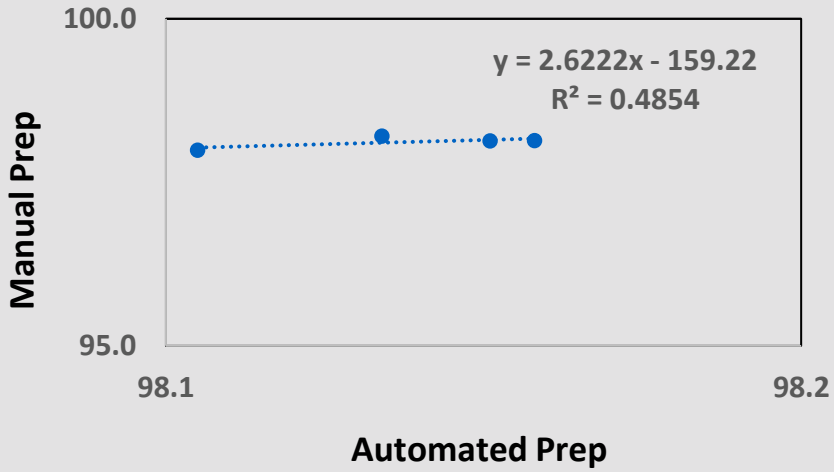
Difference in migration time may be due to different lots of consumables used and days of preparation.

MANUAL VS. AUTOMATED PREPS REDUCED CE-SDS DATA BISPECIFIC MOLECULE #1





Sample profiles are comparable, with less than 0.1% absolute difference in % Purity.



PURIFICATION SAMPLES IN 96-WELL PLATE FORMAT

Alternate Monoclonal Antibody

		Manual Prep	Automated Prep
	#	% Purity	
Sample	1	97.536	97.501
Sample	2	97.098	97.421

Fusion Protein

		Manual Prep	Automated Prep
	#	% Purity	
Sample	1	51.901	52.579
Sample	2	47.223	47.590
Sample	3	52.585	53.009
Sample	4	53.011	53.545

Bispecific Molecule

		Manual Prep	Automated Prep
	#	% Purity	
Sample	1	96.678	96.357
Sample	2	97.085	97.033

All % difference values are < 1%.

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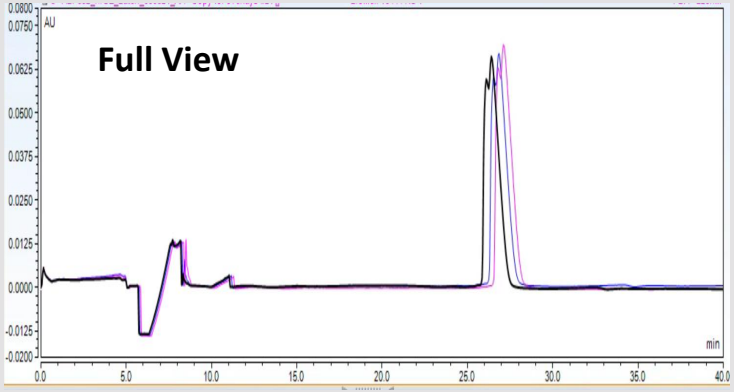
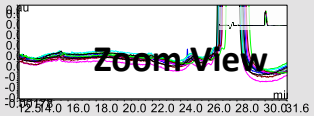
AUTOMATED SAMPLE PREP #1 EVALUATION SUMMARY

- 96-well plate format allows for higher throughput analysis
- Able to do 3 sample preparations per day
  - Three sets of 24 injections per sequence (n= 19 samples x 3)
- Able to do full preparation from start to end, or can be used to prepare buffer trays alone only
- All consumables are reusable except for pipette tips, normalization plate and reducing or non-alkylating reagent wells
- Limits exposure to hazardous alkylating reagent
- Analyst walkaway time: between 1.0 - 1.5 hours depending on number of samples
- Not a significant time savings for small number of samples (example n=4)

AUTOMATED PREP #2: MAB #1 CE-SDS PROFILES CONSISTENT ACROSS DIFFERENT 96-WELL POSITIONS

Non-reduced CE-SDS





spike

High %RSD observed for % Pre-peaks, potentially due to its level close to LOQ = 0.5%

N=12	% Pre-peaks	% Main Peak
Average	0.691	99.309
Std Dev	0.087	0.087
%RSD	12.587	0.088

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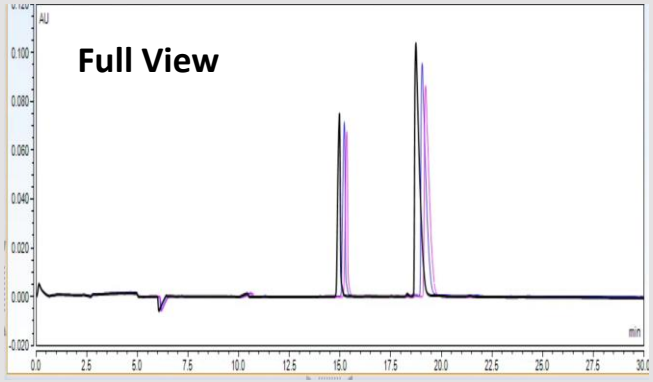
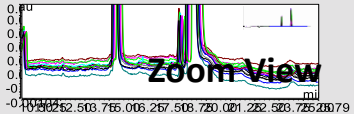
AUTOMATED PREP #2: MAB #1 CE-SDS PROFILES CONSISTENT ACROSS DIFFERENT 96-WELL POSITIONS

Reduced CE-SDS





High %RSD observed for all minor peaks due to the method's LOQ = 0.5%

N=12	% LC+HC	% LMW	% LC	% MMW	% NGHC	% HC	% HMW
Ave	99.005	0.098	30.190	0.089	0.664	68.815	0.144
Std Dev	0.118	0.038	0.108	0.080	0.020	0.094	0.033
%RSD	0.119	39.246	0.358	90.003	3.049	0.136	22.600

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AUTOMATED PREP #2 ADDITIONAL WORK

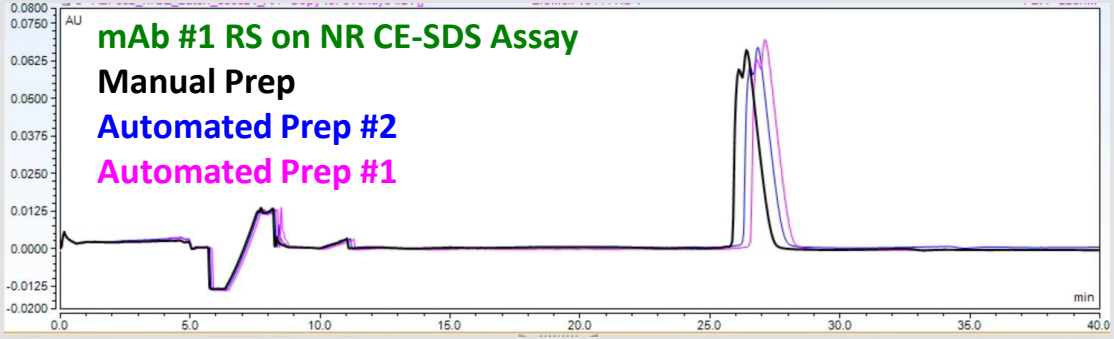
Next Steps:

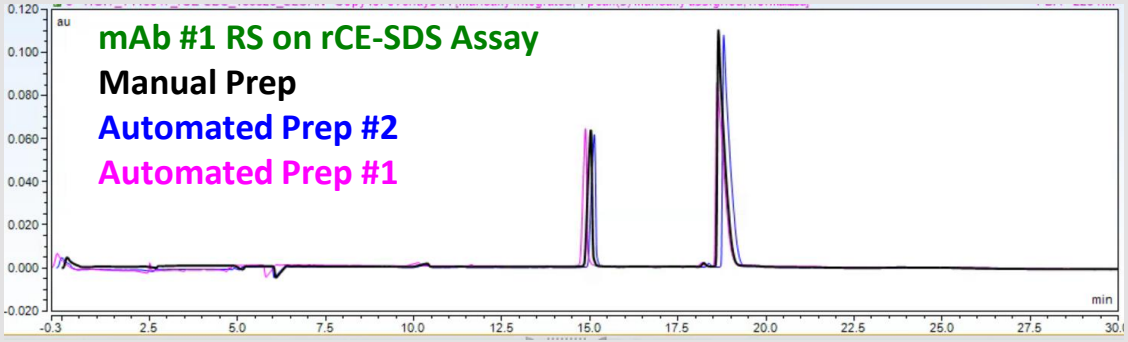
- Continue evaluation for the comparability of minor peaks
- Add custom labware definition for buffer trays
- Develop additional script for buffer vials filling

Explore full automation including sample neutralization

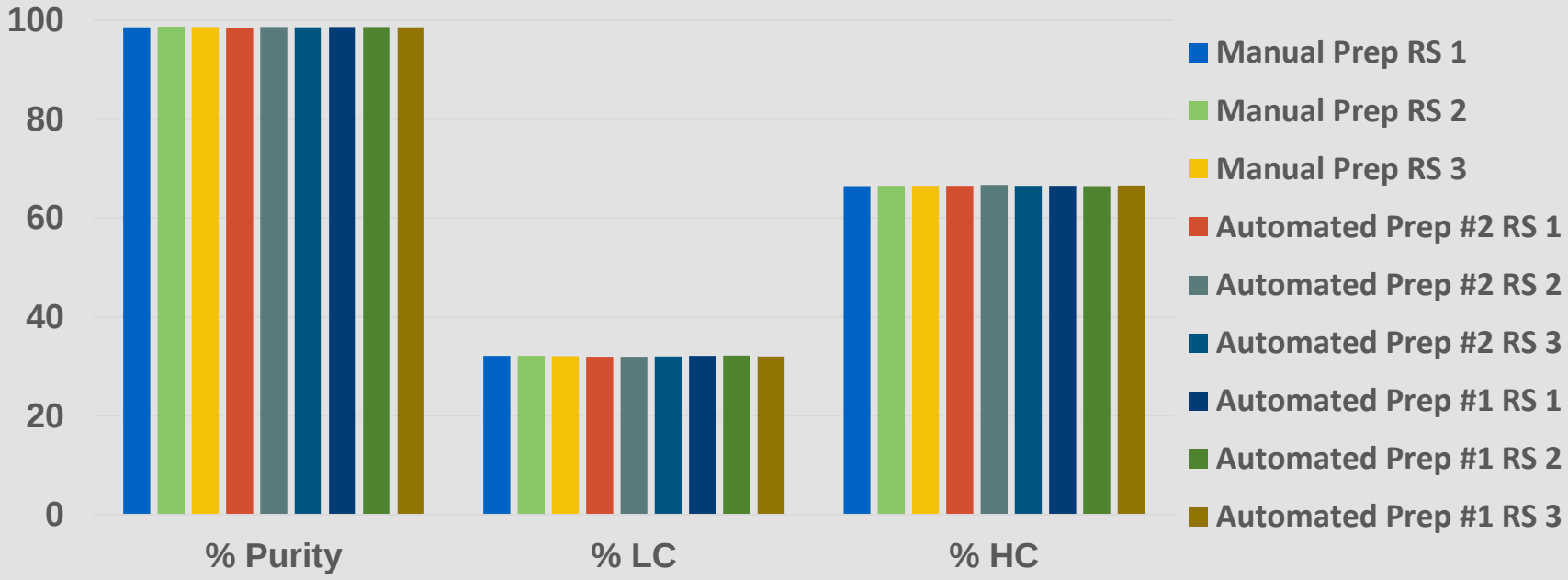
- Evaluate a higher-end automation instrument for end to end automation in principle

MAB #1 RS PROFILES CONSISTENT ACROSS THREE CE-SDS SAMPLE PREPARATIONS





MAB #2 RS SYSTEM SUITABILITY DATA CONSISTENT ACROSS THREE CE-SDS SAMPLE PREPARATIONS



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CONCLUSION

- **Automating the CE-SDS sample preparation produces repeatable results with less human errors.**
- **Both automated preps have shown to be comparable with the manual prep.**
  - **Equivalent sample profiles**
  - **Results for major peaks within 1-2% of assay variability**
- **Further evaluation is required to address the high variability of the minor species for the small liquid handler used in the automated prep #2.**

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- ProteinSimple/Bio-Techne Maurice team
- Hamilton Company Microlab Prep automation team

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BACK-UP SLIDES

AUTOMATED SAMPLE PREPARATIONS

The first integrated CE-SDS liquid handler sample preparation workflow has tremendously reduced analyst hands-on/bench time. It helps standardize daily pipetting routines with accuracy, maintains sample quality and generates repeatable, reliable results. Comparability data from several molecules consistently shows less than 1% difference when samples were prepared either manually or with the first automated prep. However, this first workstation requires a custom and costly ventilating enclosure to prevent analyst exposure to hazardous chemical fumes.

The second liquid handler for the automated prep #2 has a smaller footprint which means less bench top occupancy in already tight and allocated lab spaces; and adequately fit in standard fume hood , with no special ventilation required for hazardous chemical exposure to analysts. Efficiency gain will be recognized in analyst walk- away time, on the assumptions and calculations based on approximately 75 CE-SDS sequences per month with 3-4 runs per day. However, it is not able to do the initial sample dilution required to normalize the protein concentration.

Ergonomically, both automated preps have the advantage of removing repetitive pipetting steps which allows for a safer working environment.