Development and Optimization of a CE-LIF Method for RNA Integrity Characterization Using SCIEX 9000 RNA Kit

CE Pharm 2025
September 8, 2025

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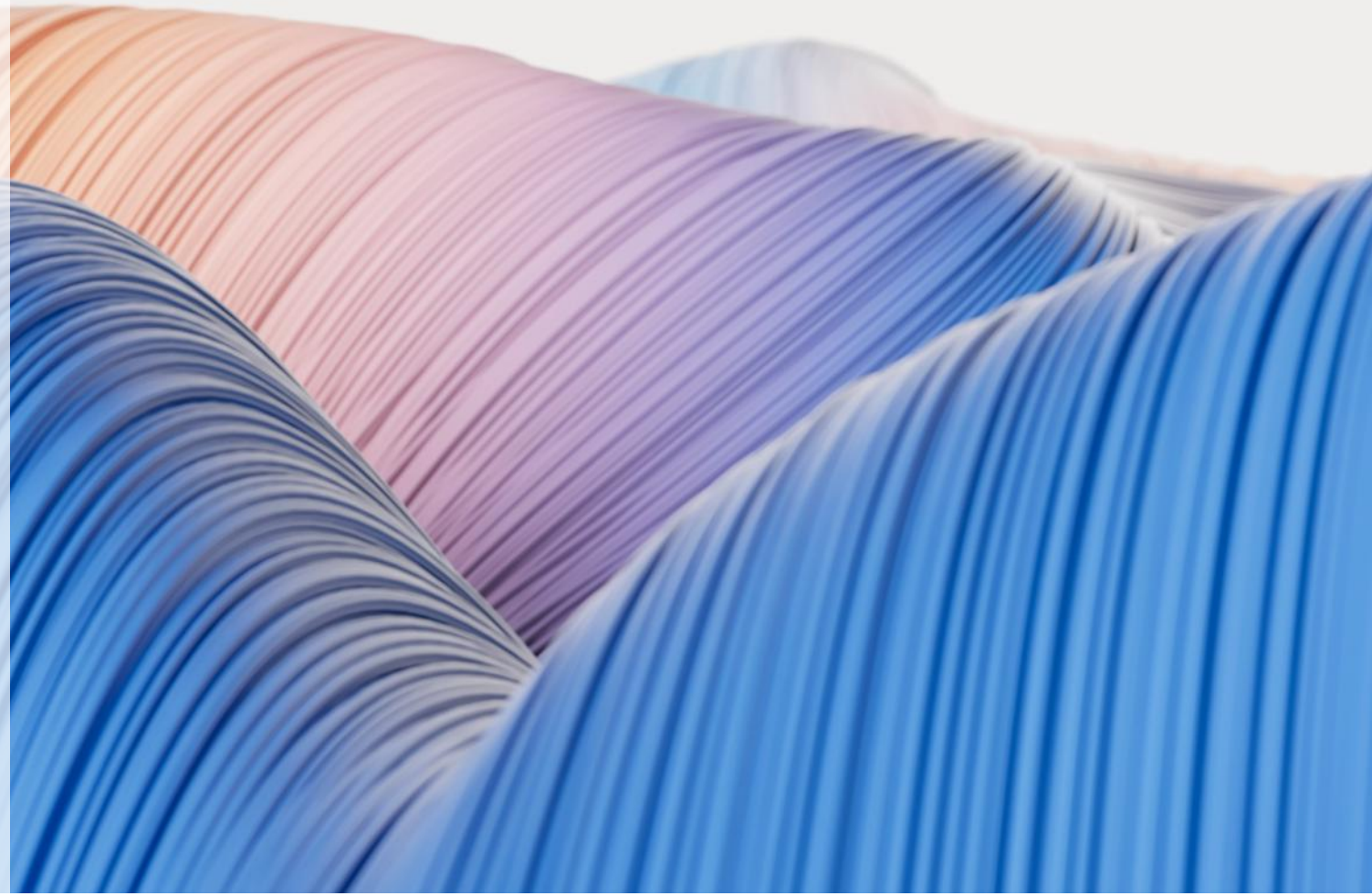


Outline

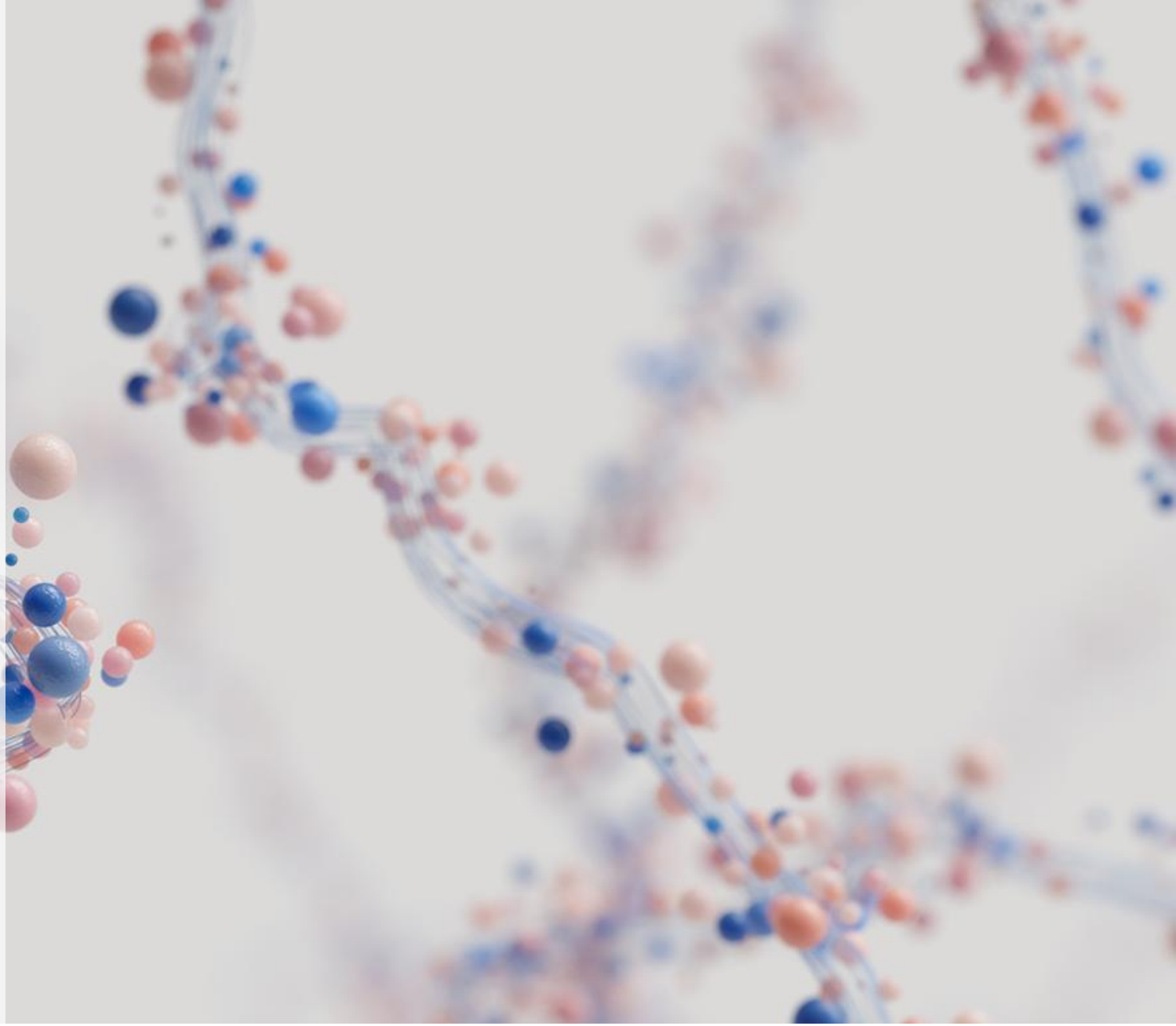
- **mRNA Vaccines and Characterization**
  - mRNA Vaccines Background
  - mRNA Integrity Characterization

- **Evaluation and Findings of SCIEX 9000 RNA Kit**

- **CGE-LIF Method Optimization & Development**
  - One factor at a time approach
  - Method transfer from PA 800 Plus to BioPhase 8800
  - Robustness evaluation

- **Conclusions**

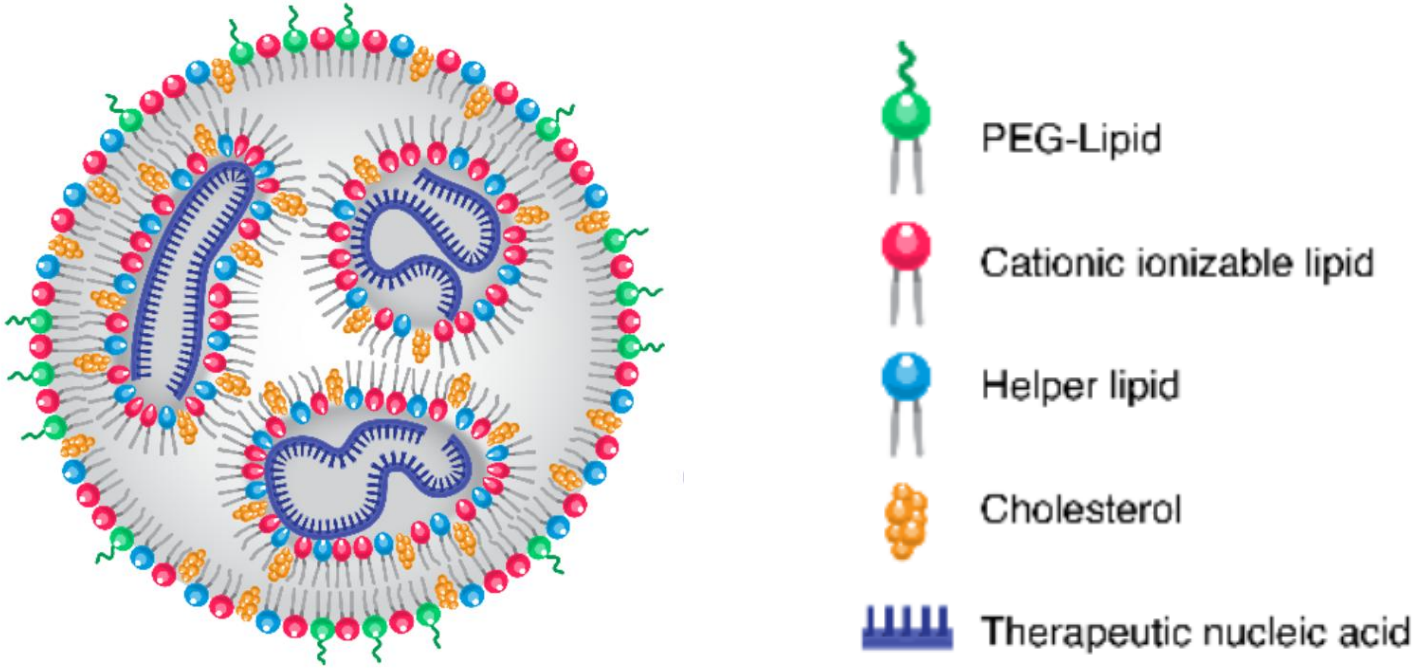
mRNA Vaccines and Characterization



mRNA Vaccines and Characterizations

RNA
Drug Substance

RNA
Lipid Nanoparticles
Drug product



mRNA Vaccines and RNA Integrity

- Pfizer has several mRNA vaccine products with a wide variety of sizes and disease targets
- RNA integrity is a critical quality attribute
  - It directly impacts efficacy, product period of use, storage conditions, and shipping tolerances
- Pfizer's release purity method is capillary gel electrophoresis (CGE) using the Agilent 5300 Fragment Analyzer

Pfizer Current RNA Integrity Method by Fragment Analyzer and Agilent RNA Kit

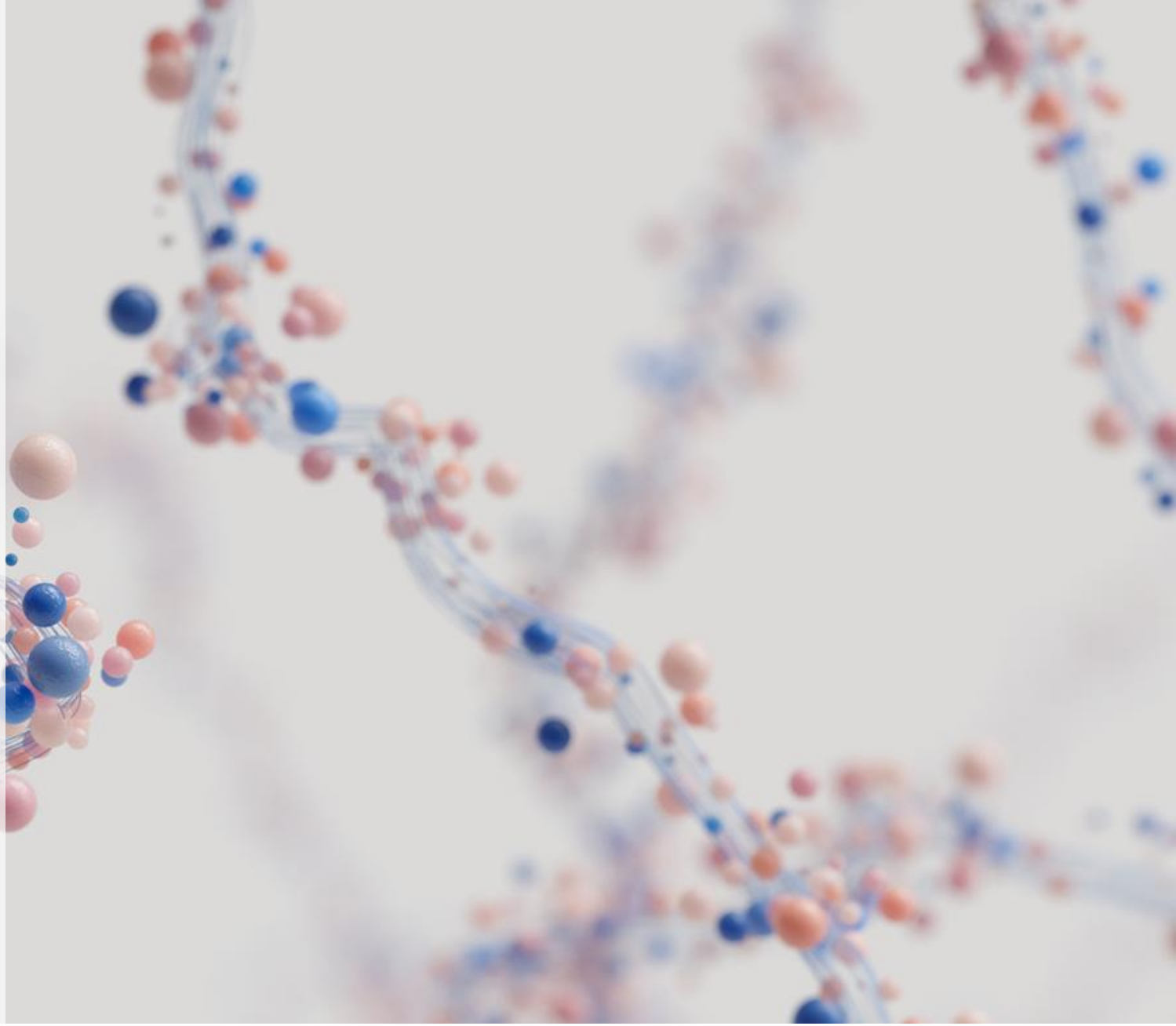
Instrument

- Agilent Multiplexed capillary electrophoresis system
  - 48 capillary array
  - Fluorescence detector
- Run time is ~1.5 hours.

Sample preparation

- **Drug substance**
  - Dilute to nominal concentration
- **Drug product**
  - Dilute to nominal concentration
  - Disrupt with detergent and alcohol solution
- In a 96 well plate, combine diluent marker (Agilent kit component that contains formamide to aid in denaturation) and sample
- Preform heat incubation for denaturation

Evaluation and Findings of SCIEX RNA 9000 Kit



Comparison of SCIEX PA 800 Plus and SCIEX BioPhase 8800 Systems

Attribute	PA 800 Plus	Biophase 8800
# of Capillaries	1	8
Modifiable Capillary	✓	✕
Capillary Coatings	✓✓	✓
Detectors	Swappable UV or LIF	Integrated UV and LIF
Ease of Automation	✓	✓✓
Empower Integration	✓	✓
Ease of Use	✓	✓✓

- Bare fused-silica capillary, 50 µm i.d., 20 cm $L_{effective}$, 30 cm L_{total}

SCIEX RNA 9000 Purity and Integrity Kit

- Sciex RNA integrity method protocol provided
- Sciex RNA 9000 Kit works for both instruments PA800 plus and BioPhase 8800
- Bare fused-silica capillary cartridge (50µm i.d. x 30 cm length)
- Bare fused-silica capillary relies on capillary coating steps

Component List

- Acid Wash /Regenerating Solution (0.1 M HCl)
- CE Grade Water
- LIF Performance Test Mixture
- ssRNA Ladder 0.05 - 9 kb
- Nucleic Acid Extended Range Gel
- Sample Loading Solution (SLS)
- SYBR Green II RNA Gel Stain (with excitation at 488 nm, emission at 520 nm)

SCIEX RNA 9000 Purity and Integrity Kit – Protocol Highlights

Methods

Condition at 20 °C
- Pressured rinse water/HCl/water for 12 min
- Gel rinse for 10 min
- Gel fill separation at 6.0 kV, 20 min.

Separation at 30 °C
- Pressured rinse HCl/water for 2 min
- Gel rinse for 5 min, pre-voltage at 30 kV for 2 min
- Electrokinetic injection at 1 kV, 3 seconds
- Separate at 6.0 kV for 22 min

Rinse at 20 °C
- Pressured rinse water/HCl/water for 12 min

Preparation

Sample
- RNA diluted with SLS/ water to 0.05 – 50 µg/mL
- Heat the sample at 70 ºC for 5 minutes.
- Cool on ice for a minimum 2 minutes.
- Sample compartment temperature is set to 10 °C.

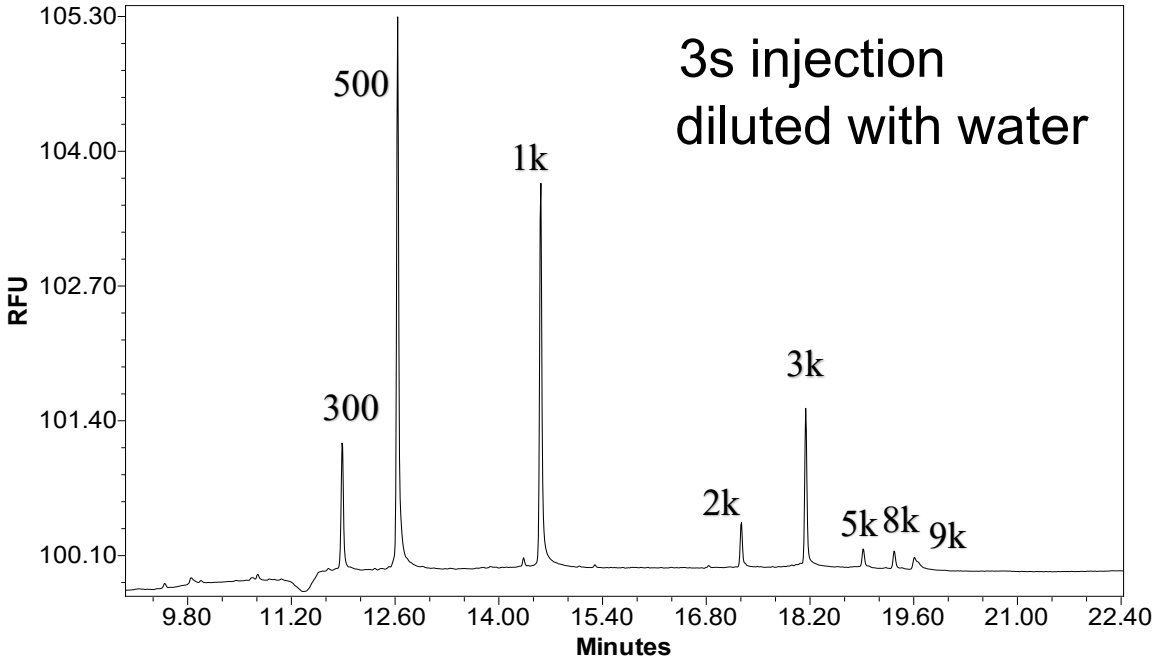
Gel buffer
Nucleic acid extended gel / SYBR green dye
= 10 mL / 20 µL

SCIEX RNA 9000 Kit Protocol Evaluation Targets

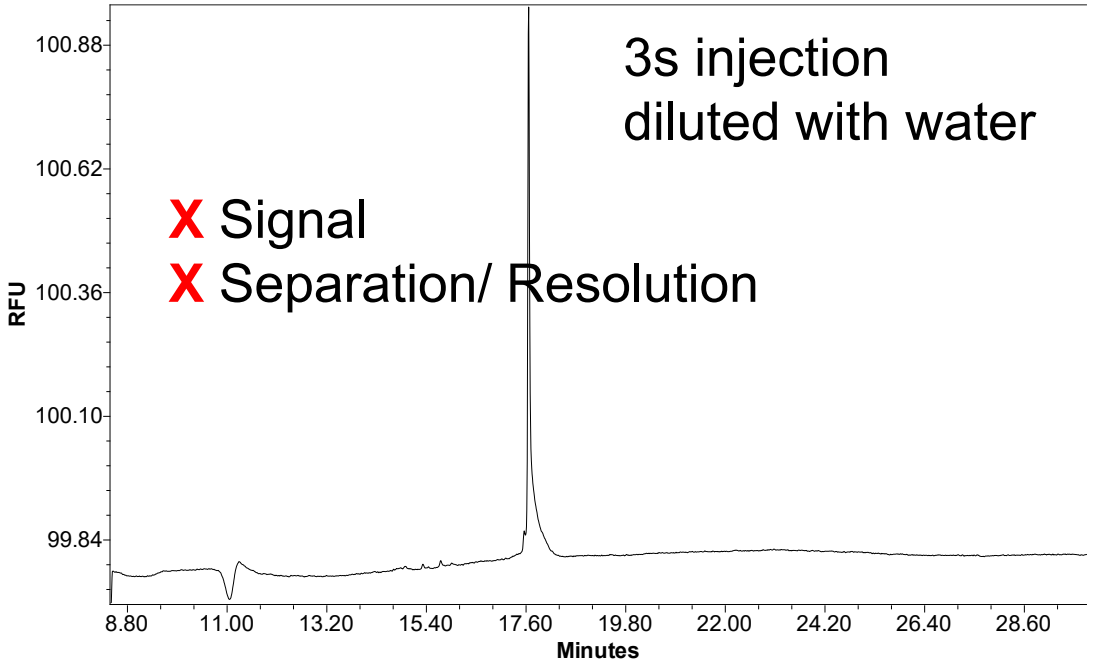
- Low noise and flat baseline for ease of integration
- Peak separation/ resolution
- Reproducibility
- Comparable result to Pfizer current RNA integrity method
- Capillary lifetime/ durability

SCIEX RNA 9000 Kit using PA 800 Plus Evaluation Result

- SCIEX ssRNA Ladder



- RNA DP, 0.5 µg/mL

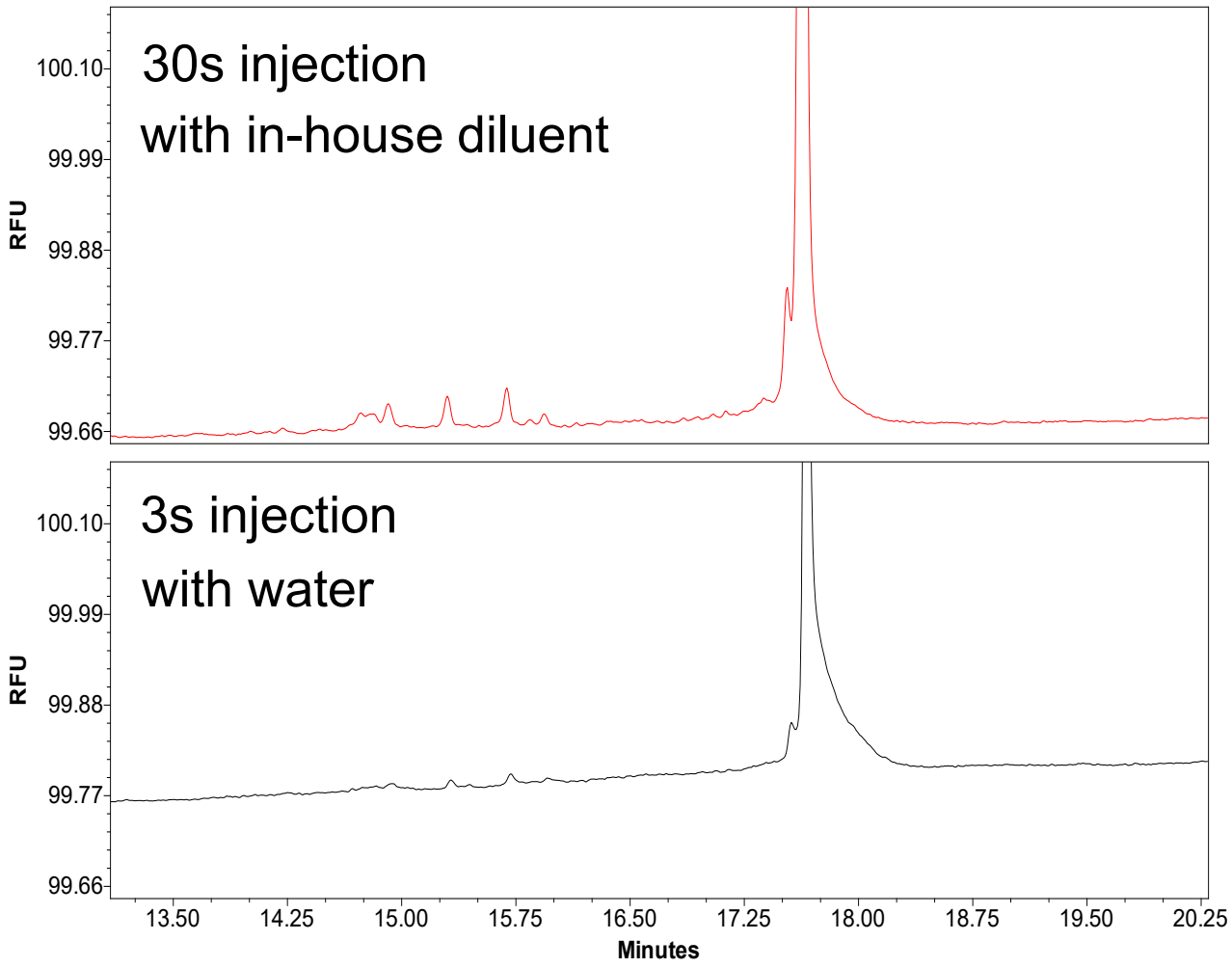


Conclusion: Low signal and resolution with 0.5 µg/mL with 3s injection duration requires further optimization

SCIEX RNA 9000 Purity & Integrity Kit for the PA 800 Plus Evaluation Result – Diluent Impact

- RNA DP loaded in 0.5 µg/mL
- Increased electrokinetic injection duration
- With in-house diluent

Zoomed-in Profile



Conclusion: Diluent helps peak separation and resolution.

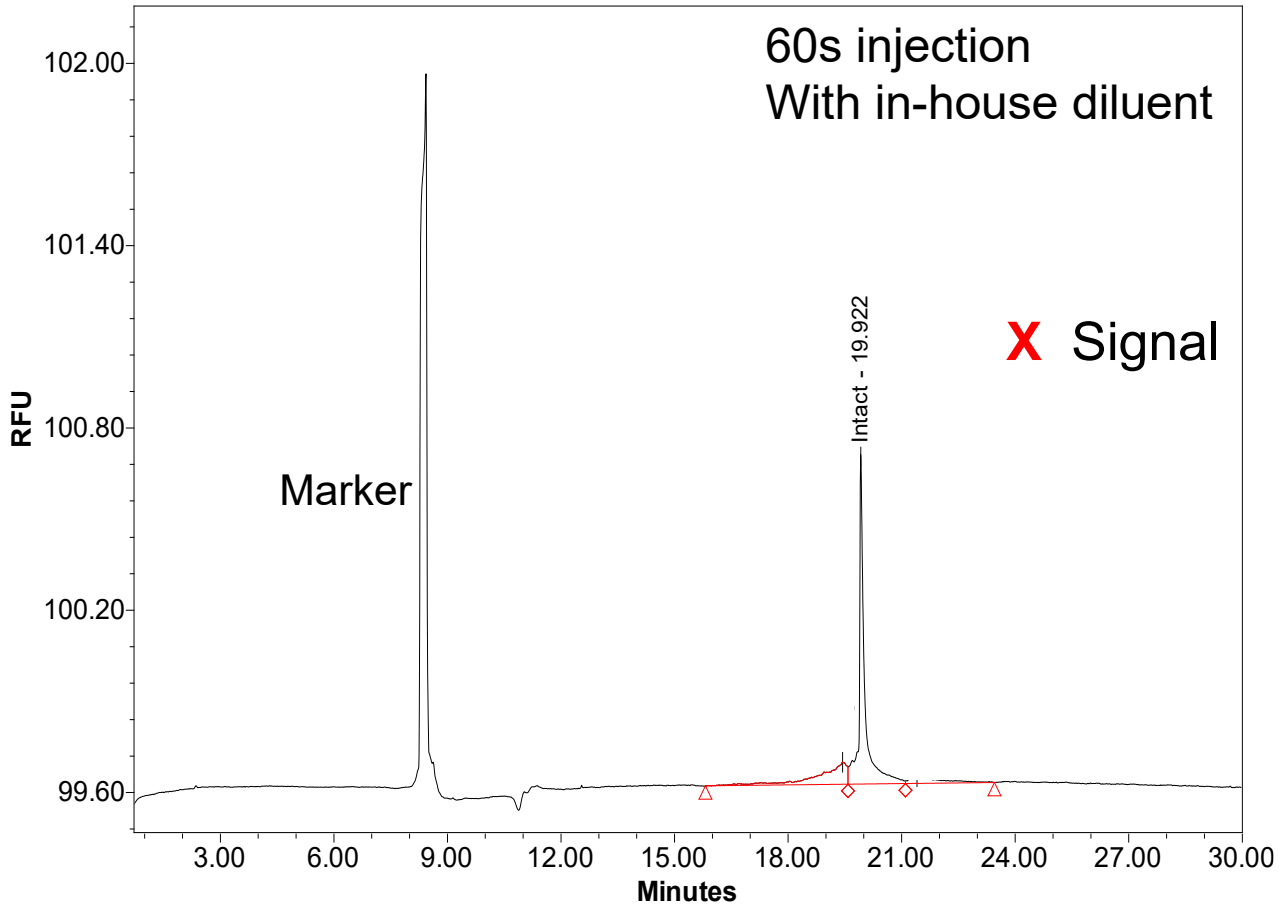
SCIEX RNA 9000 Purity & Integrity Kit for the PA 800 Plus Evaluation Result – Repeatability

➢ RNA DP loaded in 0.5 µg/mL

RNA DP	% Intact	Total TCA
Preparation 1	--	318
Preparation 2	--	287
Preparation 3	--	180
Ave	**--**	**262**
%RSD	**6.3%**	**27.7%**

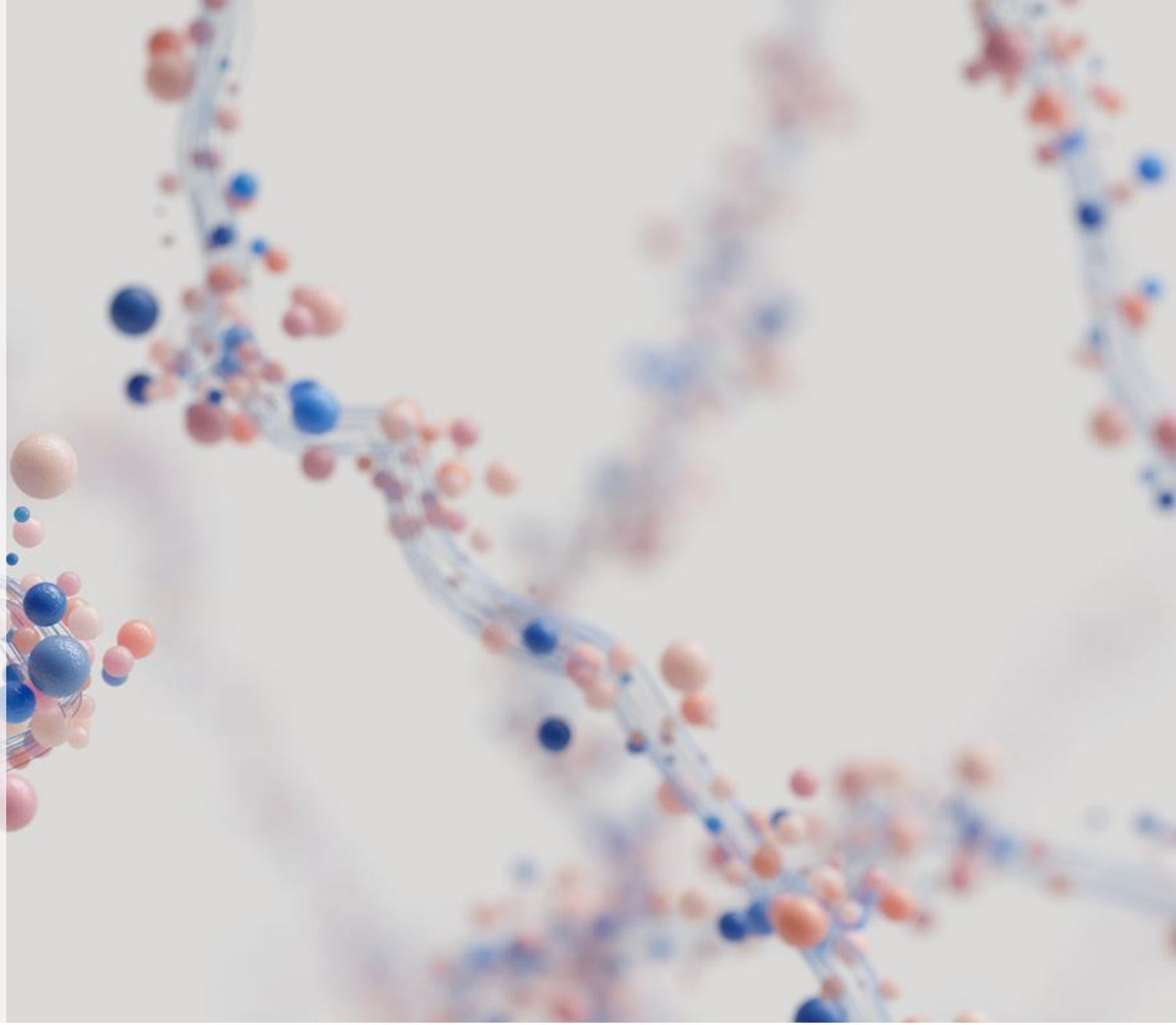
X Intact% repeatability (N=3)

Full Profile



Conclusion: The signal and repeatability need to be improved.

CE-LIF Method Optimization and Development for RNA Integrity Characterization



How to Increase Sample Injection Signal

- Optimize sample electrokinetic injection
  - Increase sample loading concentration
  - Adjust injection time duration
  - Adjust injection electric voltage

$$Q = (u_e + u_{eo})E\pi r^2 C\mathrm{t}$$

Injected amount

Electrophoretic mobility

Electro-osmotic mobility

Electric field strength

Capillary radius

Concentration

Injection time

- Dye amount in the gel

- Sample diluent

Sample Injection Signal Optimization

- **Diluent screening study**: vendor diluents & in-house diluents
- **Sample injection mechanism and conditions**: pressure vs. electrokinetic (1 kV vs. 5 kV)
- **Gel buffer/ dye:** 20 µL dye/10 mL gel; increase dye amount in the gel

Optimized condition summary

- Electrokinetic injection at 5 kV
- Increased dye amount in the gel buffer
- Optimized a sample diluent

Lesson Learned

New Capillary Conditioning

- Recommend condition 2-3x prior to first use

Capillary Lifetime

- No basic NaOH rinsing possible
- Store capillary at 5 °C after use
- High temperature/high voltage may decrease use period

Choice of evaluation material

- Recommend RNA DS (remove formulation variable from initial method development)
- Assess mRNA-LNP as second step

How to improve the peak separation/resolution based on PA800 plus

- **Separation voltage**

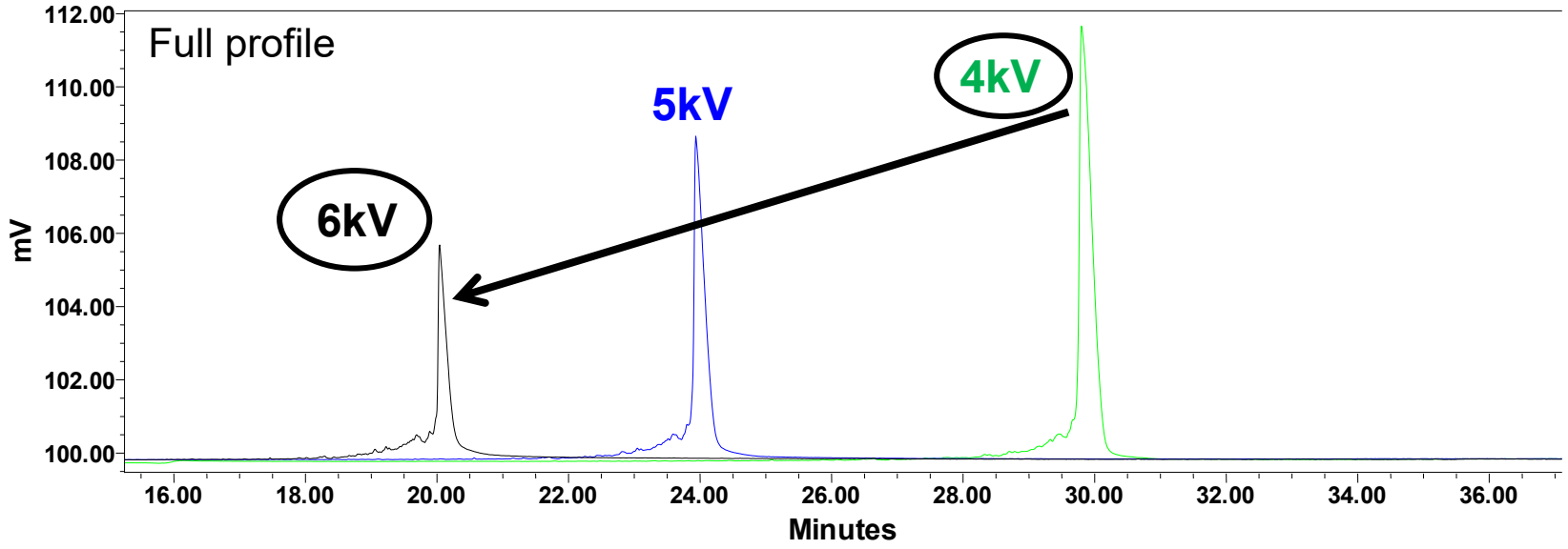
Separation voltage impact 4 kV, 5 kV and **6 kV**

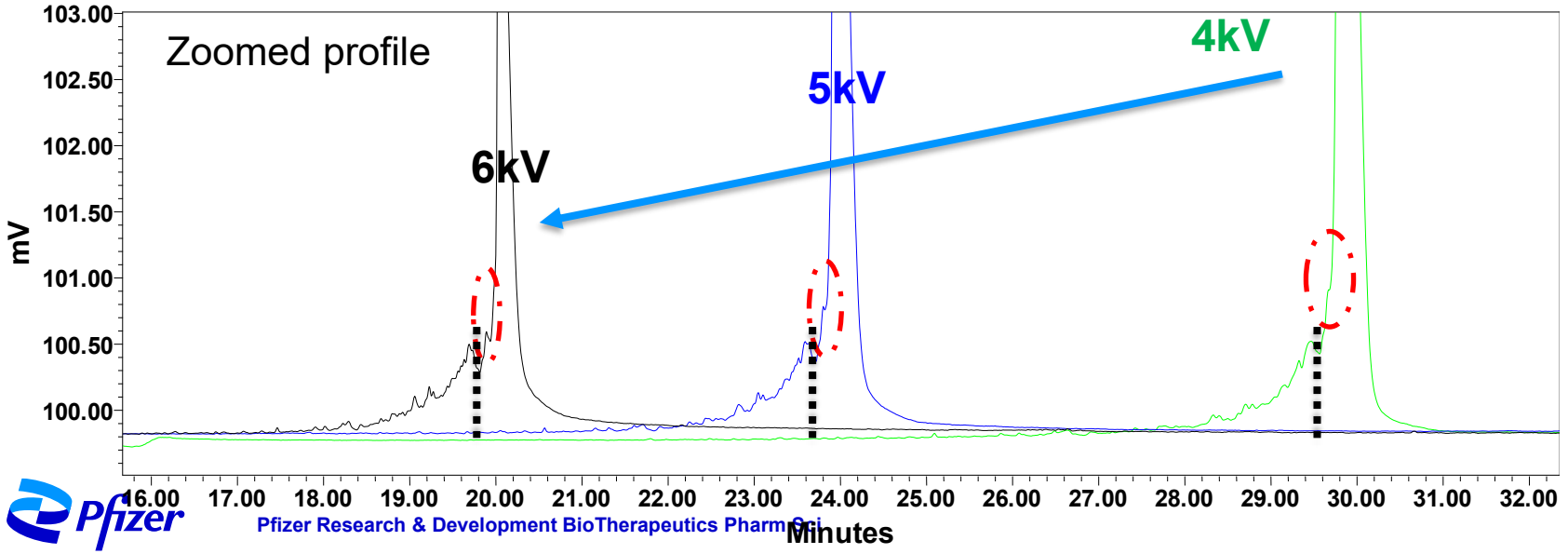
- **Temperature**

Capillary temperature impact 25 °C, **30 °C** and 35 °C

Separation Voltage Study

- RNA DS at 6, 5, 4 kV separation voltage evaluation with temperature at 30 ºC

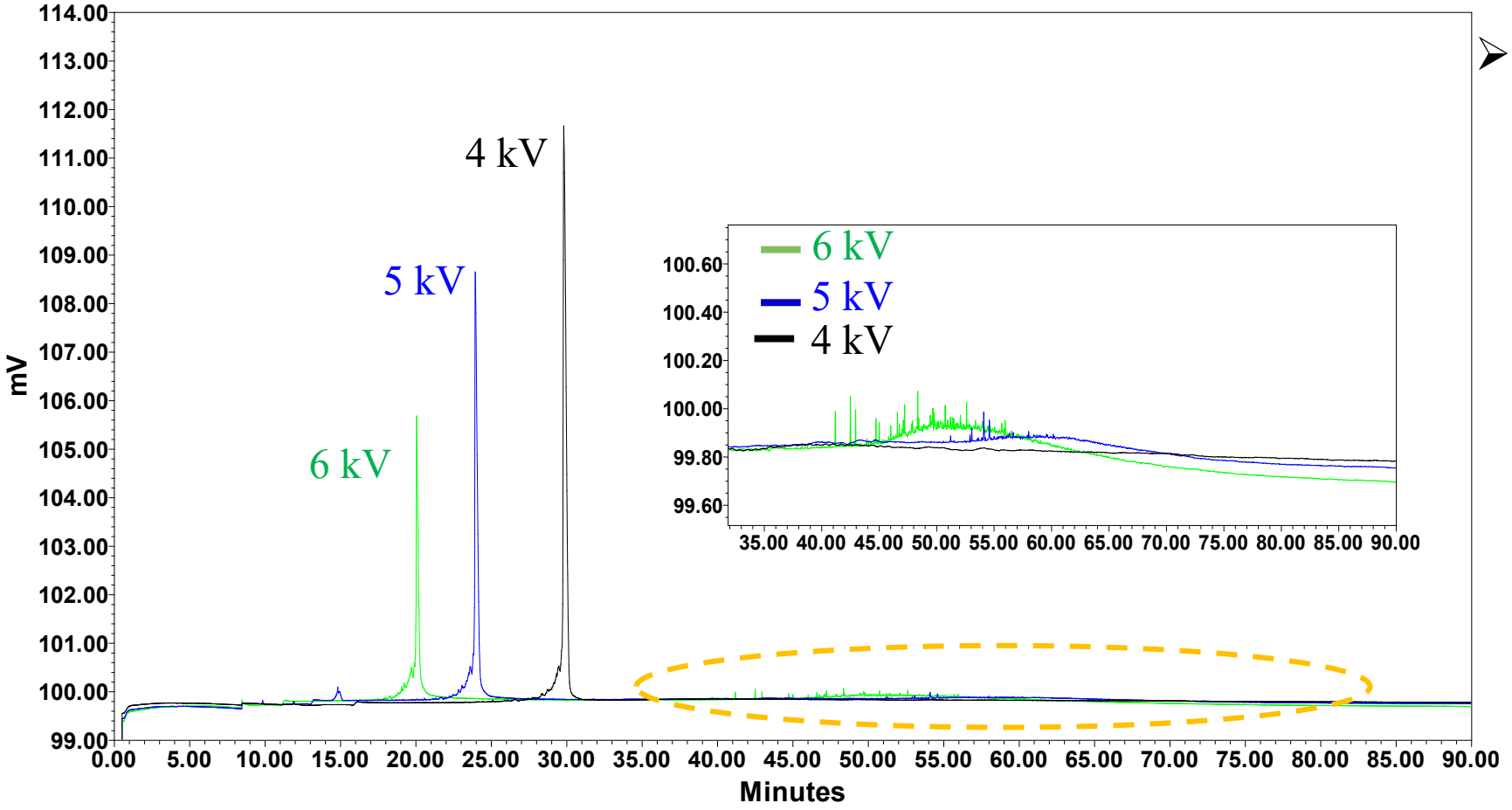




- Total TCA decreasing from 4kV to 6kV:
  - Residence time in detection window
- % Intact decreasing from 4kV to 6kV:
  - It's possible that voltages produce more resolved fragments yield different integrity values compared to those with lower resolution

Separation Voltage Study

- "Unknown" noise peaks observed for separation voltage at 6 kV and 5 kV



➢ At 4 kV separation voltage

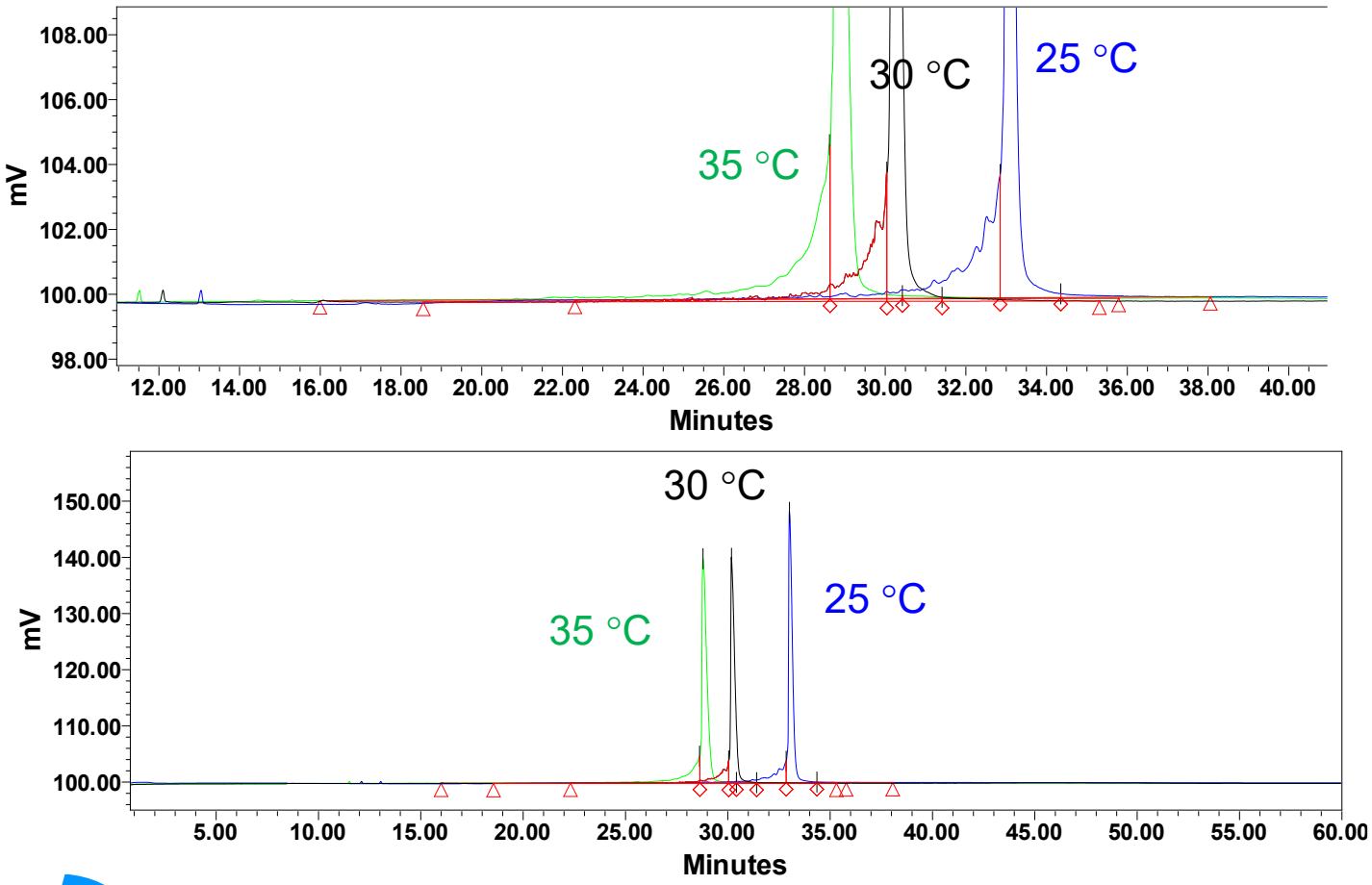
- No "unknown" peaks observed
- Consistent repeatability for RNA % intact
- Comparable % intact to Pfizer current FA method

Conclusion: Separation voltage 4 kV is chosen.

Separation Temperature Study

- RNA DS at 25, 30 and 35 °C capillary temperature evaluation with separation voltage at 4 kV

- Separation/resolution
- In-capillary stability



- 35 ºC the peaks lost the resolution/separation
- 25 ºC and 30 ºC the peak profiles are comparable.

RNA DP Lipids Disruption

- Screening study

- Lipids disruption with detergent and alcohol solution:

- Evaluate different organic with & without different detergents

- Lipids disruption condition evaluation:

- Incubation (time/temp.) vs. vortex (time at room temp.)

- RNA DS will be used as control

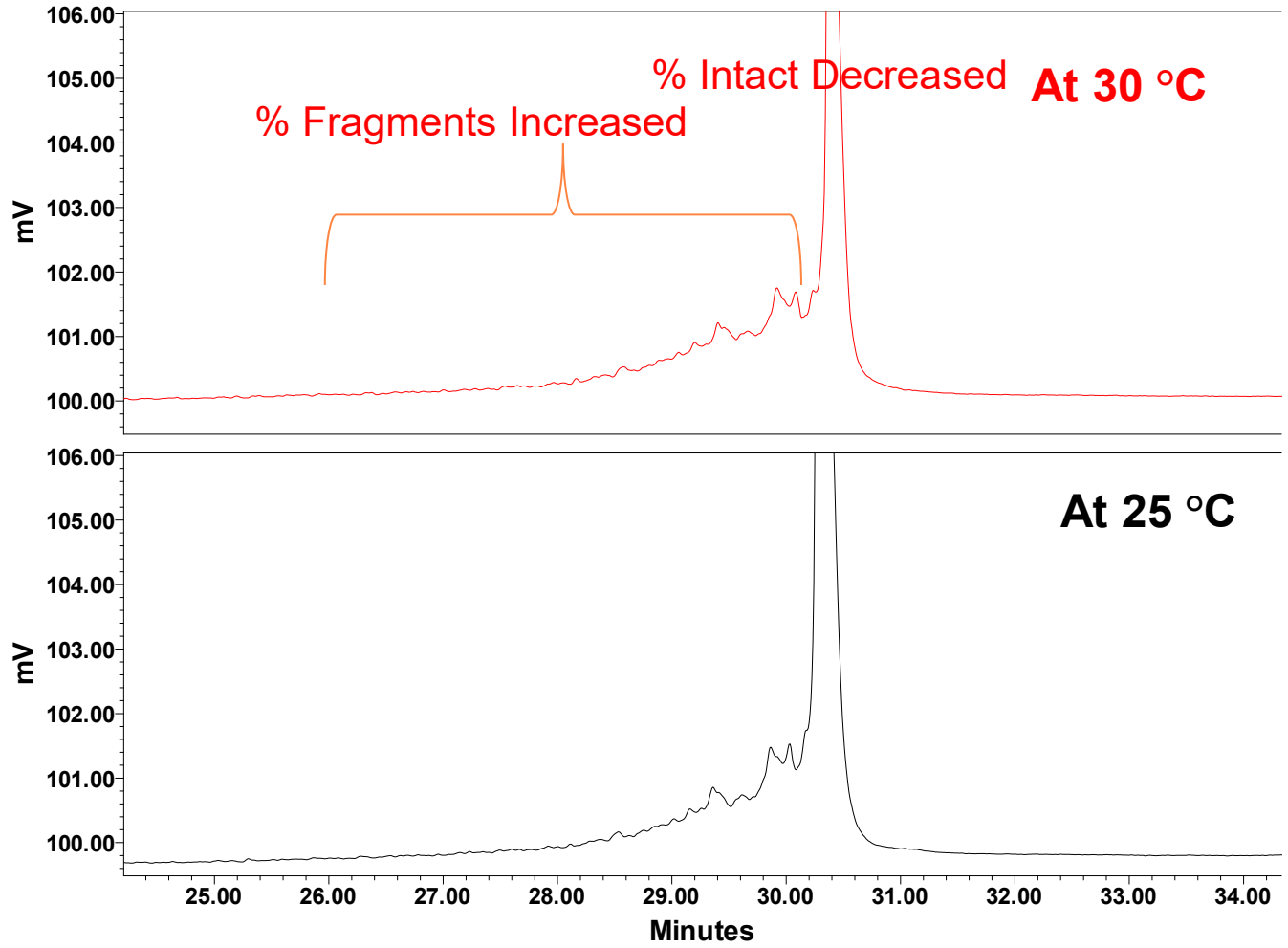
- To monitor lipid disruption condition impact on the RNA stability.

Lipid disruption summary

- Lipid disruption detergent/alcohol matrix determined
- Heat the sample at 70 ºC for 5 minutes
- Cool on ice for a minimum 2 minutes

RNA DP Separation with Capillary Temperature 25 and 30 °C Evaluation

- RNA DP shows in-capillary degradation under 30 ºC.



- Comparing to 25 ºC, at 30 ºC RNA % intact is decreased significantly.

Conclusion: 25 ºC is chosen

RNA DP Robustness Evaluation using PA800 plus

- ➢ 6 hours on-board at 10 ºC autosampler
  - RNA integrity decreased within 3% RSD
  - Total TCA decreased within 5% RSD

- ➢ Repeatability (N=18): 2 analysts, 2 capillaries, 3 runs
  - Percentage integrity: % RSD = 3
  - Total TCA: %RSD = 12

RNA DP Robustness Evaluation using BioPhase 8800

- The data are comparable from PA800 plus to BioPhase 8800

- Repeatability (N=24), 8 capillaries, 3 hours on board
  - Percentage integrity: % RSD = 3
  - Total TCA %RSD = 8

- Each Single capillary repeatability (N=3)
  - Percentage integrity: % RSD = 1- 6
  - Total TCA: %RSD = 2 - 9

Conclusion

- CGE-LIF method has been optimized based on SCIEX 9000 RNA kit
- The method is repeatable between the PA800 Plus and Biophase instruments with comparable electropherograms and percent RNA integrity
- Capillary durability under current optimized condition is ~80 injections
- Lack of capillary regeneration procedure

Acknowledgments

Pfizer

Andover
Dave Ripley
John Davis
Benjamin Vincent
Austin Degroot
Shauna Salem
Qian Wang
Tanya Shang

St. Louis
John Orlet
Christopher Hood
Gaofei He
Yan He

SCIEX

Eric LaRoche
Ted Amazeen