Long Term Precision of Fast CZE of mAb and Fast CGE of Plasmids with Uncoated Capillary

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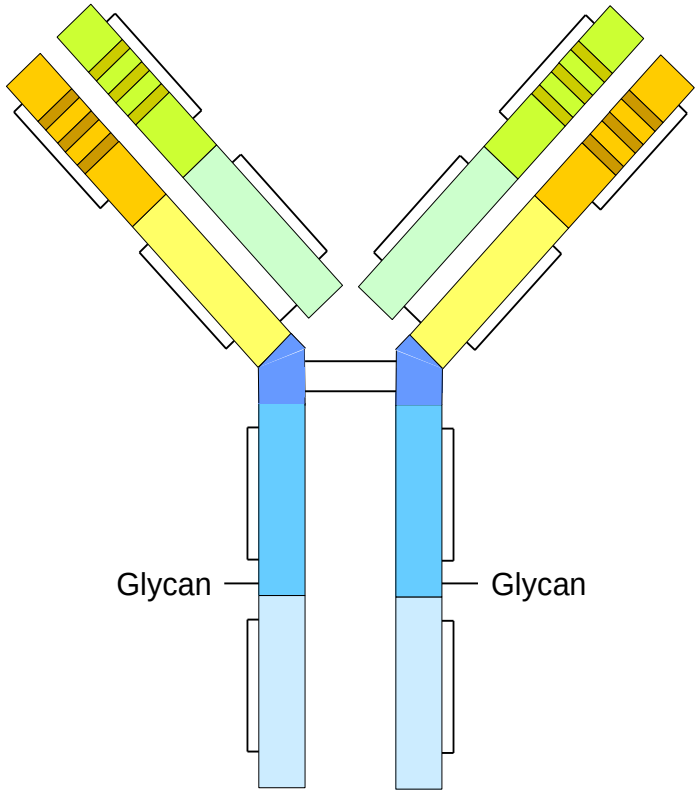
Pfizer WORLDWIDE RESEARCH, DEVELOPMENT AND MEDICAL

Overview

1. mAb structure and charge variants
2. Techniques for analysis of mAb charge variants
3. Rapid Analysis of mAb charge variants by CZE-UV with uncoated capillary
4. Long term precision and capillary lifetime of CZE-UV of mAb charge variants

5. Plasmid DNA structure and topoisomers
6. Rapid Analysis of plasmid topoisomers by CGE-LIF with uncoated capillary
7. Long term precision of CGE-LIF of plasmid DNA topoisomers
8. Summary

mAb Structure and Charge Variants



MW ca. 150K

#	Degradation Pathways	Effect	Charged Variants
1	Deamidation	new COOH	acidic
2	Sialylation	new COOH	acidic
3	C-terminal Lys cleavage	loss of NH2	acidic
4	Cysteinylation	conformational	acidic
5	N-terminal glutamine cyclization	loss of NH2	acidic
6	Maleuric acid adduct	loss of NH2	acidic
7	Glycation	loss of NH2	acidic
8	Thiosulfate adduct	new acidic group	acidic
10	Succinimide formation	loss of COOH	basic
11	Asialylation	loss of COOH	basic
12	C-terminal Lys amidation	loss of COOH	basic
13	Oxidation	conformational	basic
14	Disulfide mediated	conformational	basic

https://doi.org/10.2174/1389201087867864O2

Techniques for mAb Charge Variants Analysis

Technique	Principle of Separation
CEX	surface charge
CIEF or iCE	Isoelectric point
CZE	Charge to size

mobility → $\mu_{ep} = \frac{q}{6\pi\eta r}$ ← charge, ← size

CZE of mAb with coated vs uncoated capillary

CZE with Permanently Coated Capillary

Mire-Sluis AR (ed): State of the Art Analytical Methods for the Characterization of Biological Products and Assessment of Comparability. Dev Biol (Basel). Basel, Karger, 2005, vol 122, pp 49-68.

Analysis of Protein Therapeutics by Capillary Electrophoresis: Applications and Challenges

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CZE with uncoated Capillary

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Analysis of Identity, Charge Variants, and Disulfide Isomers of Monoclonal Antibodies with Capillary Zone Electrophoresis in an Uncoated Capillary Column

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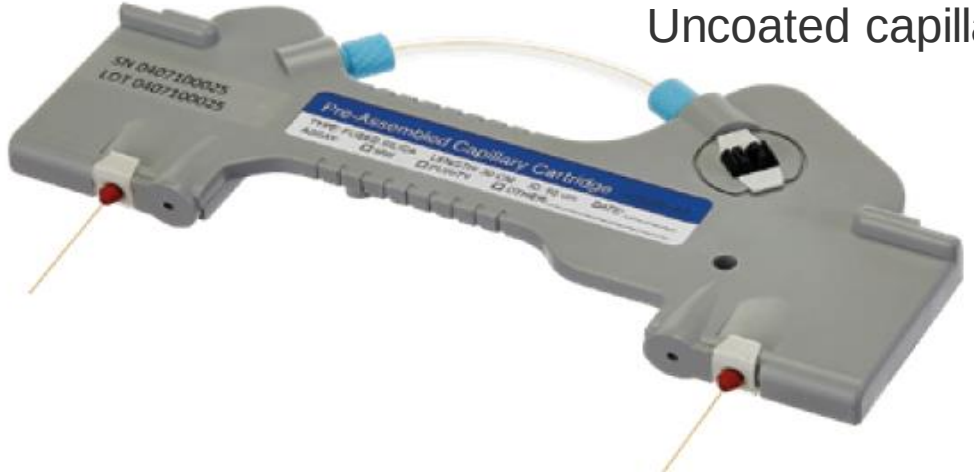
SCIEX CZE Kit for mAb separation with uncoated capillary

0.1 M HCl

water

CZE Buffer

Uncoated capillary



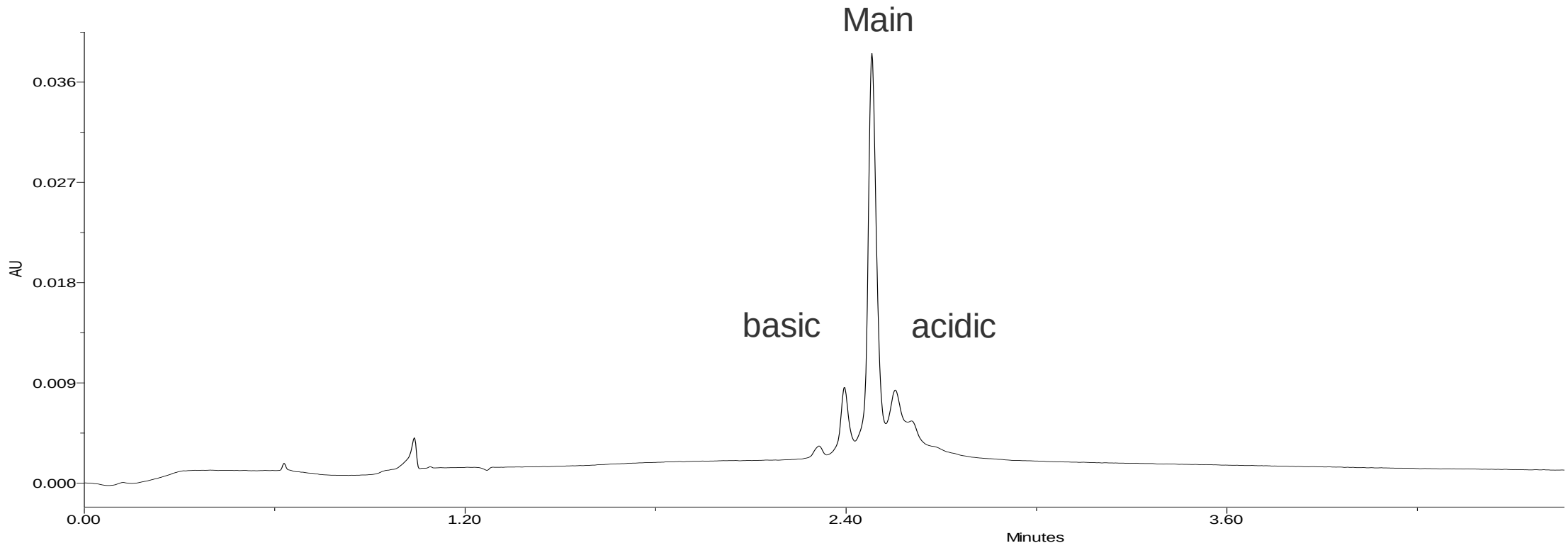
Factors Affecting Capillary Lifetime and Long Term Precision

1. BGE (Buffer, pH, additives)
2. Capillary cleaning
3. Capillary storage
4. mAb properties (pI, hydrophobicity)
5. mAb loading

Different Capillary Storage Conditions

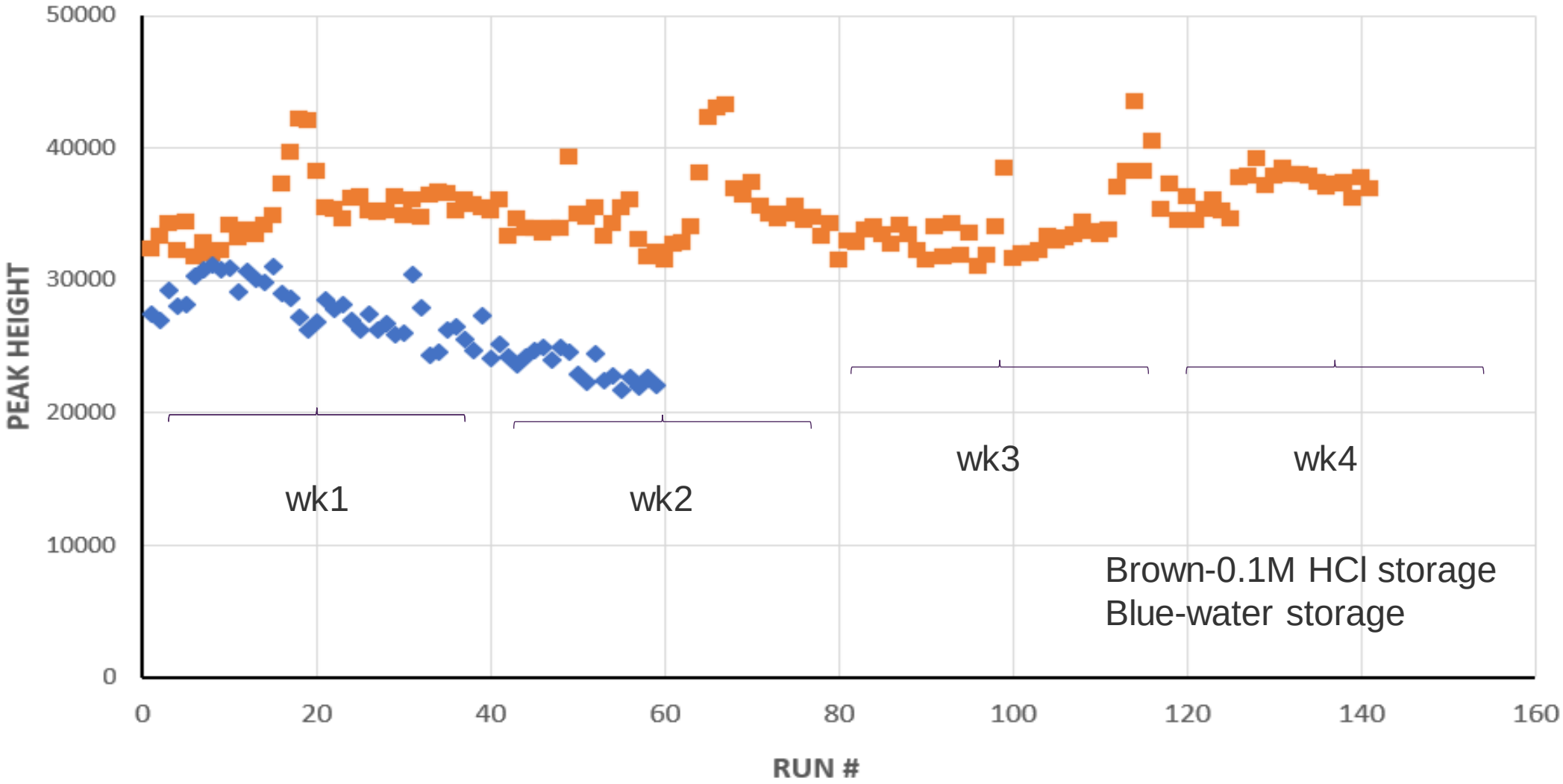
1. Separation buffer
2. 10 mM phosphoric acid+ water
3. 0.1 M HCl + 1 mM phosphoric acid
4. 0.1 M HCl + water
5. Water –recommended in CZE kit

Rapid Analysis of RituximAb with CZE-UV



Buffer: SCIEX CZE buffer, 0.1 M HCl flushing: 50 psi x 1min, Buffer flushing: 50 psi x 1 min.
Capillary: (10+20) cm x 50 um id
Vinj: 0.3 psi x 8 sec, Vsep: 22 kV
Sample: 1 mg/ml RituximAb (Sigma, Cat# **MSQC17**)
Capillary storage: 0.1 M HCl + water

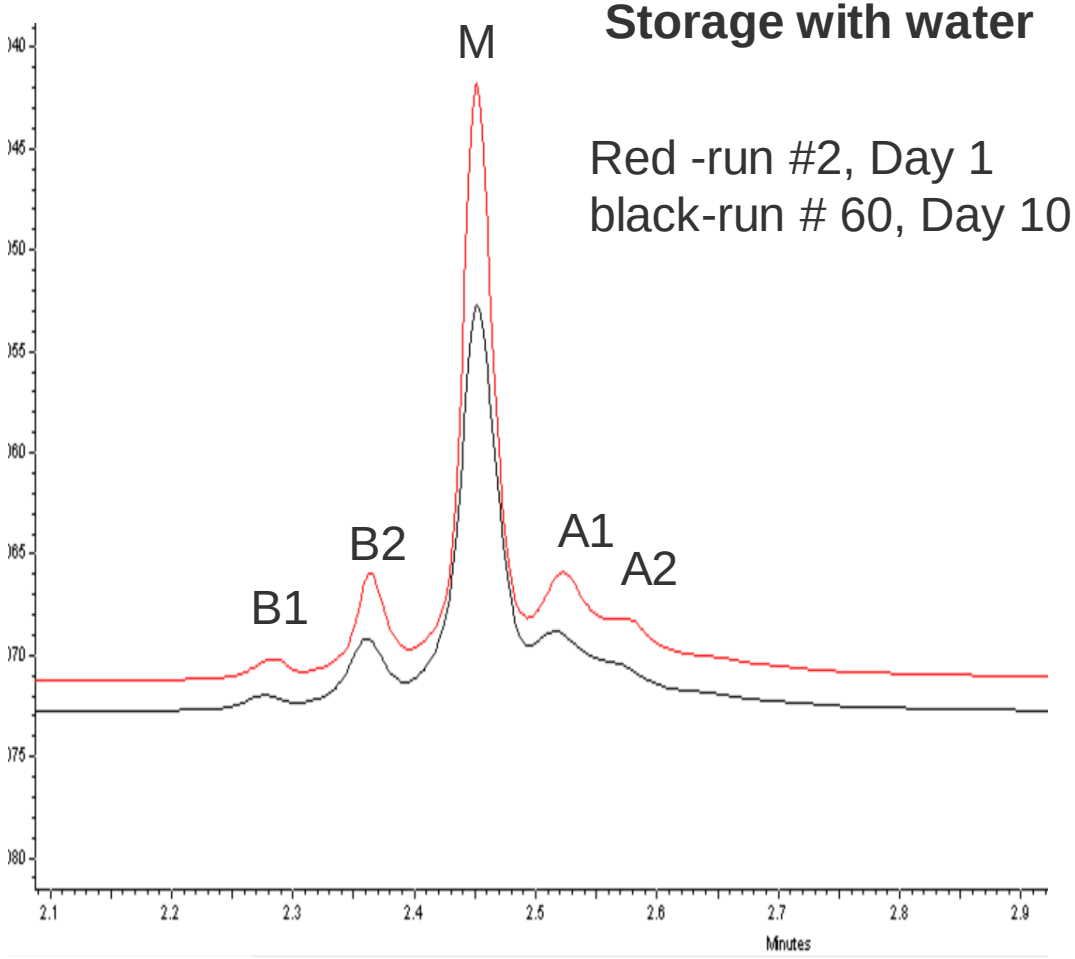
Long Term Variability of Main Peak Height



Comparison of Separation and Capillary Lifetime

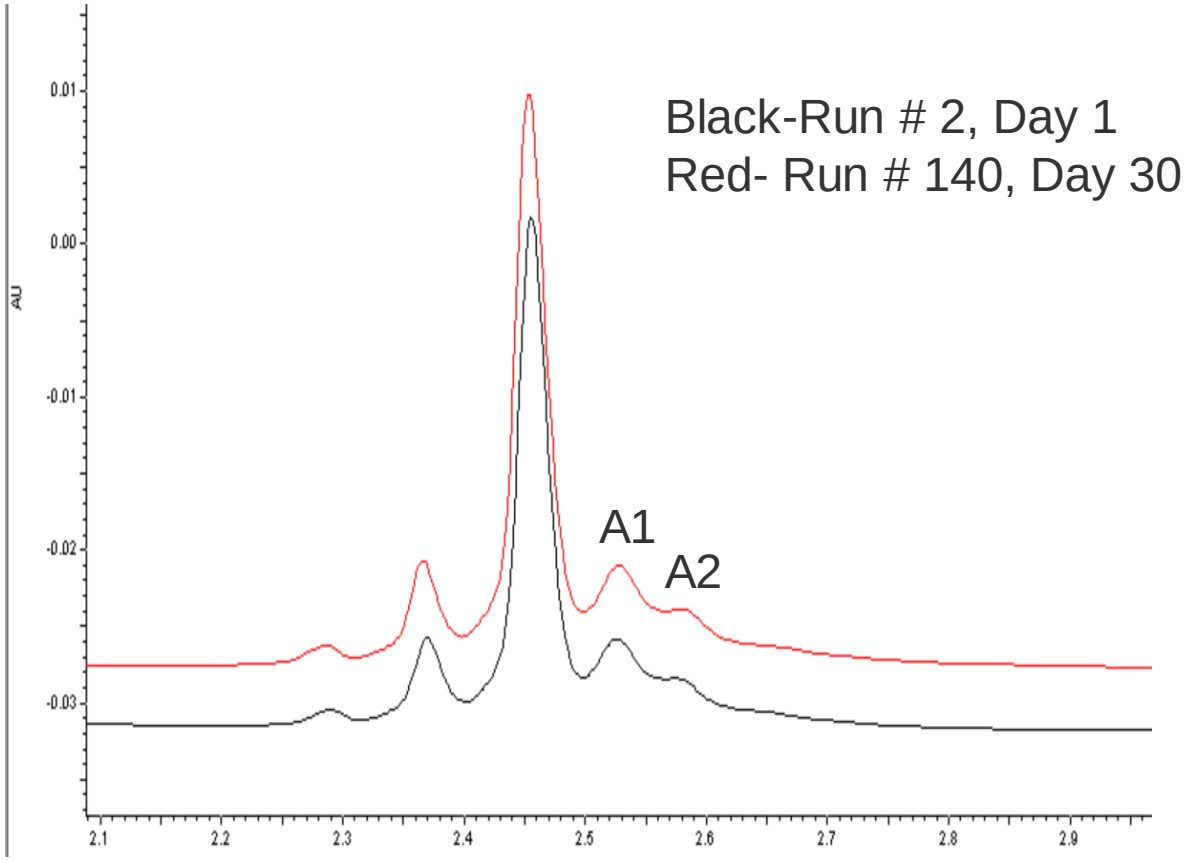
Storage with water

Red -run #2, Day 1
black-run # 60, Day 10

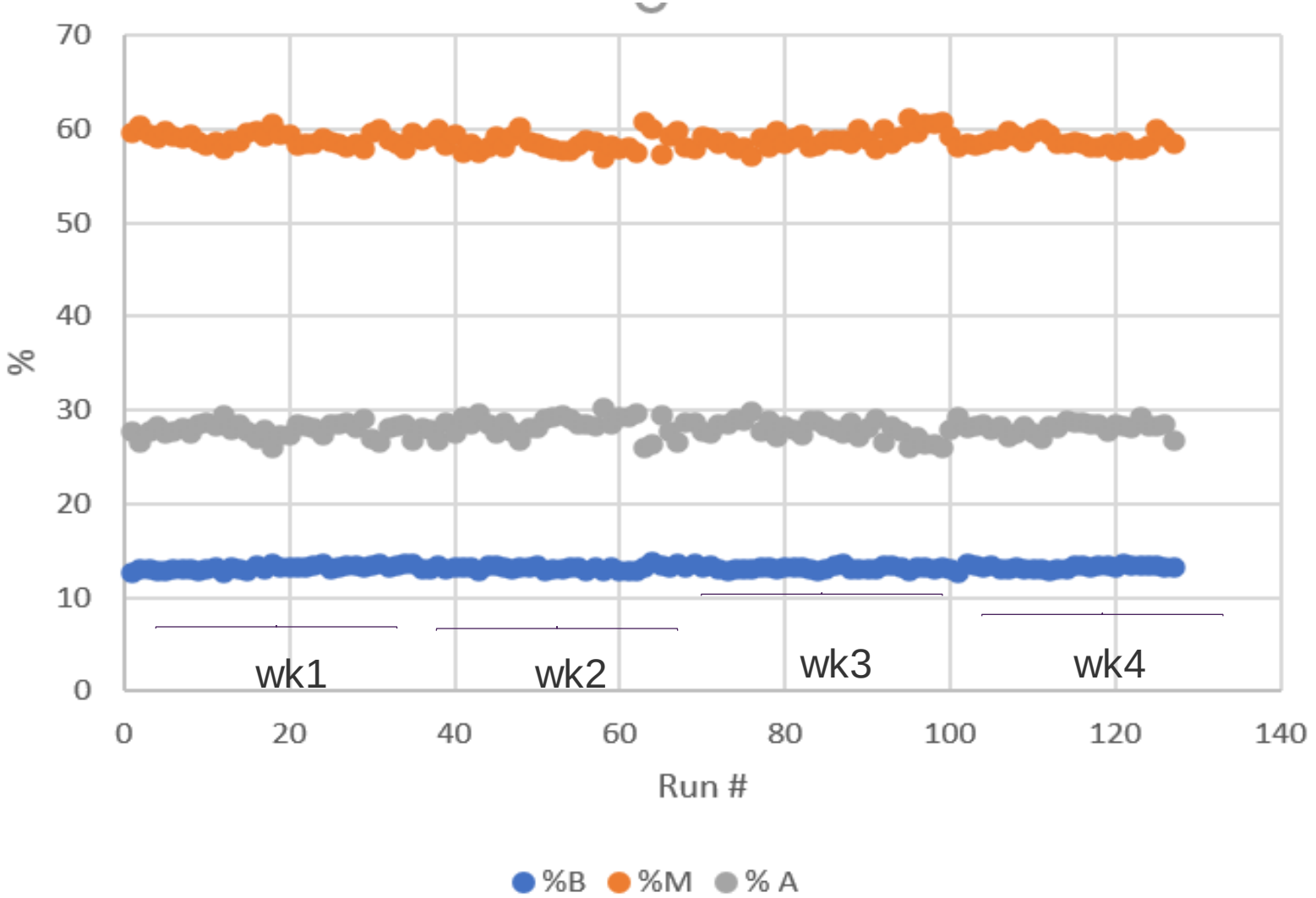


Storage with 0.1M HCl

Black-Run # 2, Day 1
Red- Run # 140, Day 30

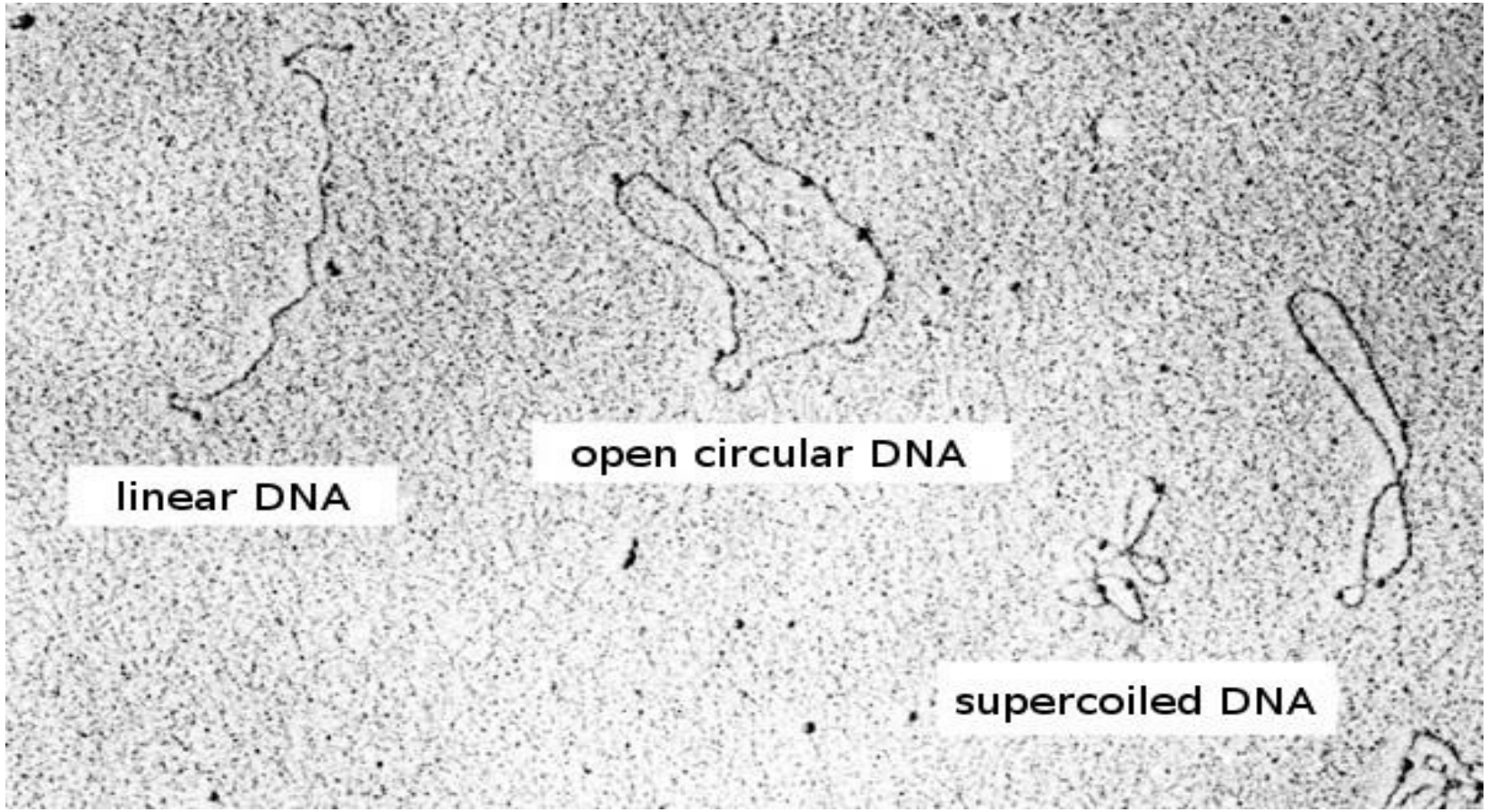


Long Term Precision of Main, Basic and Acidic %



	Basic%	Main %	Acidic %
SD	0.22	0.84	0.88
mean	13.2	58.8	28.1
RSD%	1.70	1.43	3.15

CGE-LIF of Plasmid DNA Topoisomers



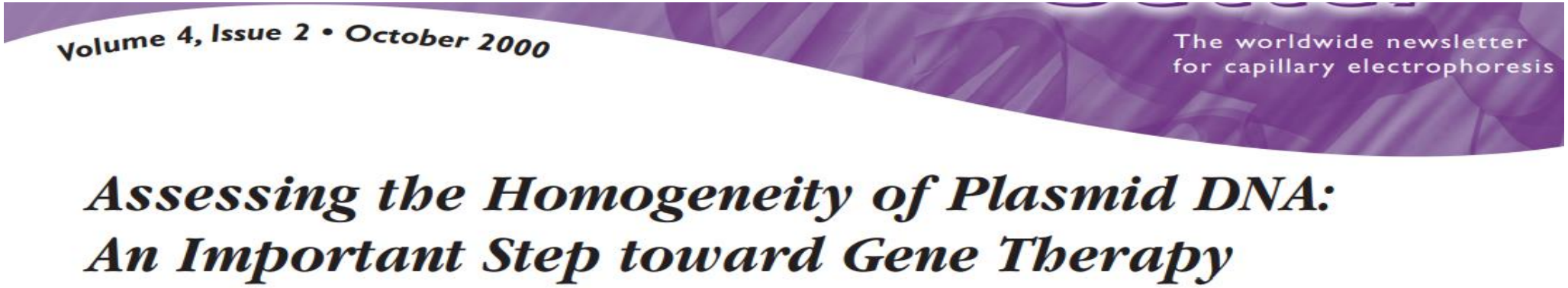
SC level: affect transfectability and stability
Regulatory Requirement: Supercoil DNA > 80%

Techniques for Plasmid Topoisomer Analysis

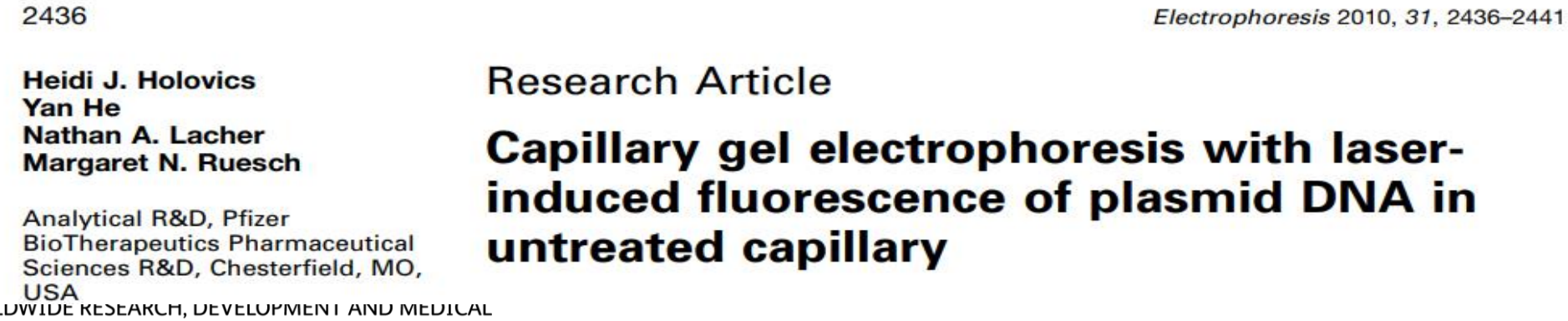
Technique	Separation Principle
AGE	conformation and hydrodynamic size
AEX	conformation and surface charge
CGE	conformation and hydrodynamic size

CGE-LIF of Plasmid Topoisomers with Coated vs Uncoated capillary

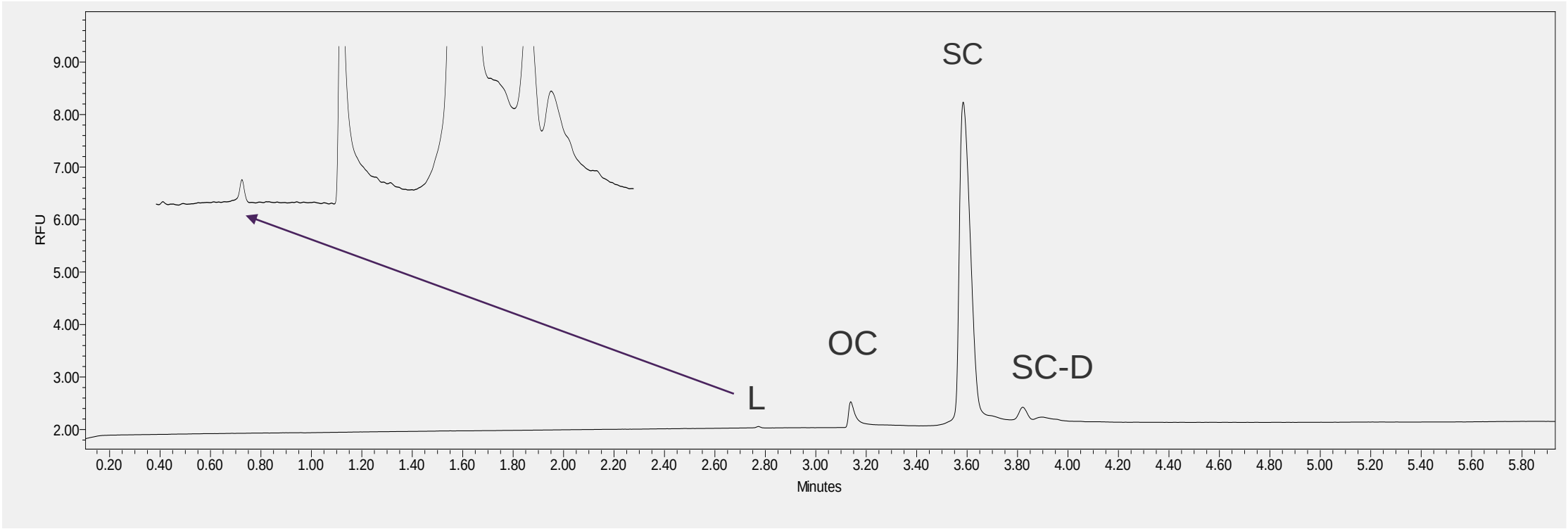
CGE-LIF with Coated Capillary



CGE-LIF with uncoated Capillary



CGE-LIF of Plasmid Topoisomer with uncoated capillary



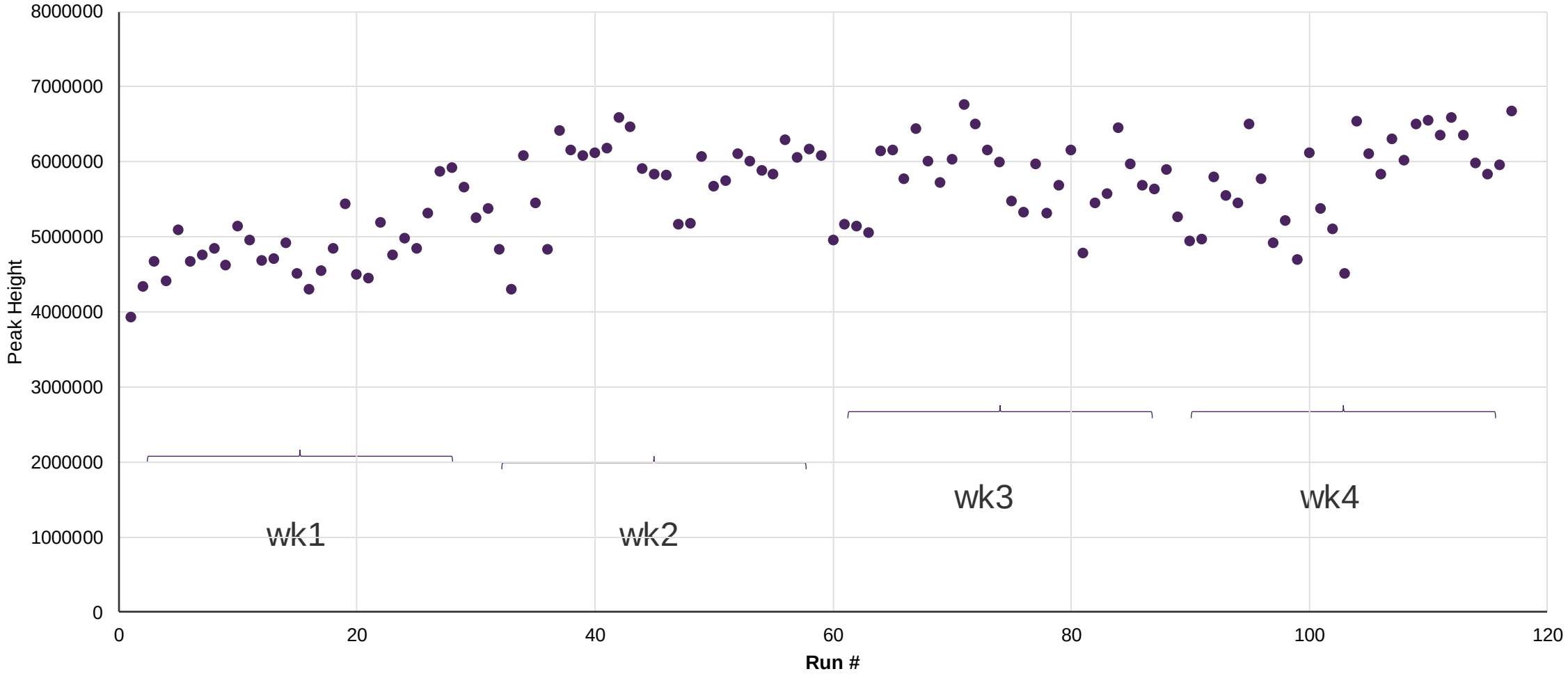
Gel buffer: 50 mM ACES, pH 6.25, 0.45% HPMC, 1 ppm EtBr, 1 mM EDTA,

Capillary: (10+ 20) cm x 50 um id, Voltage: - 10 kV, Vinj: -2 kV x 8 sec, Plasmid con: 10 ug/ml,

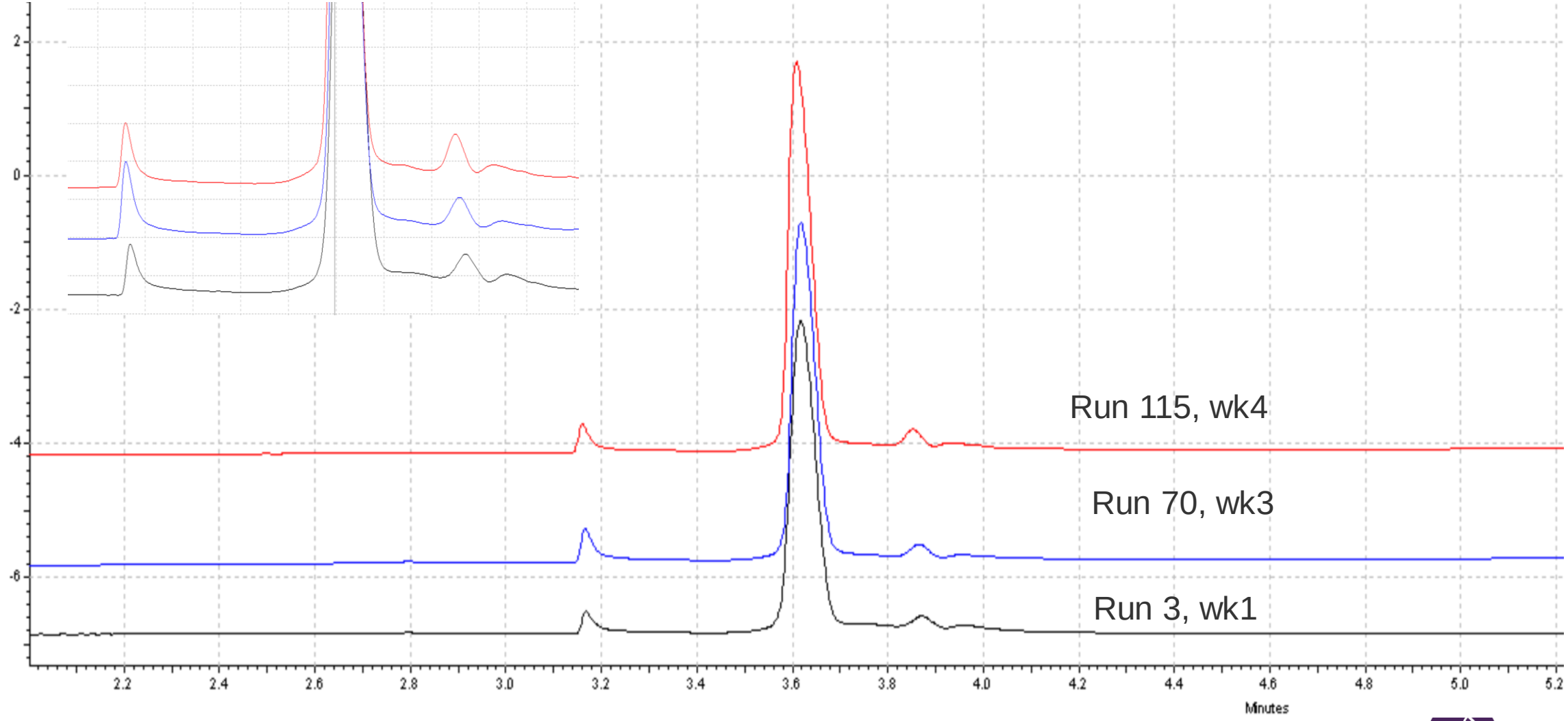
Excitation: 488 nm, Emission : 605 nm

Capillary storage: 0.1 M HCl+water

Long Term Tracking of SC Peak Height

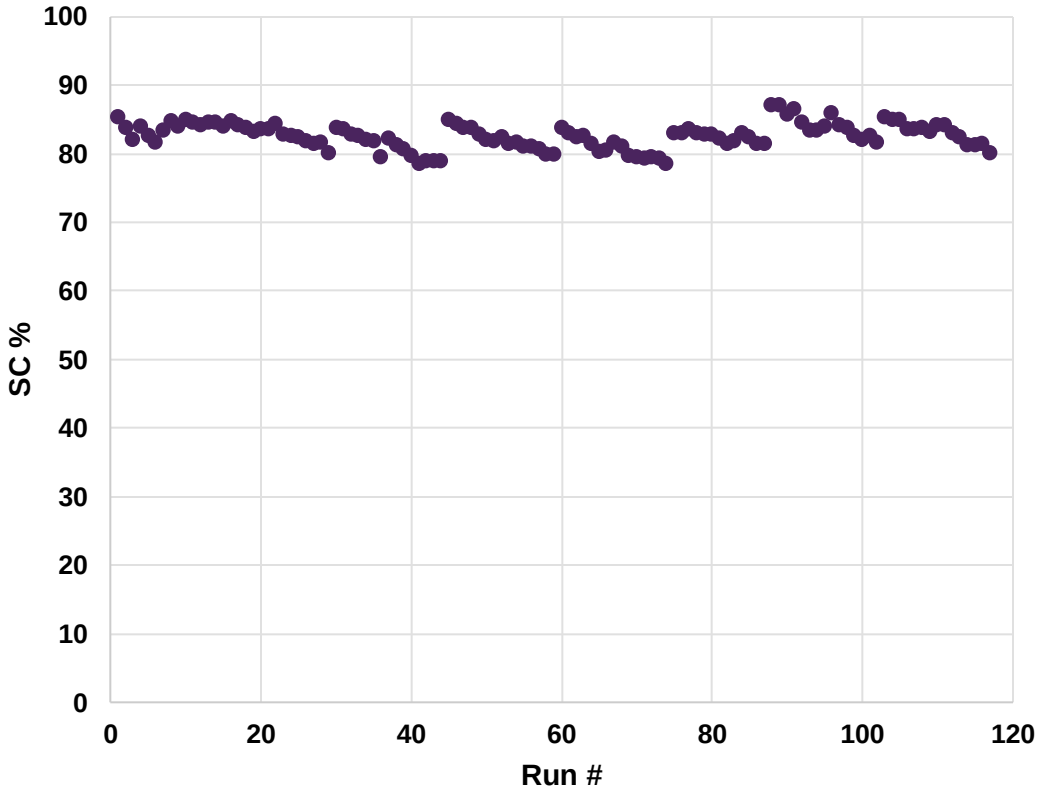


Comparison of Separation at Run 3, 70 and 115

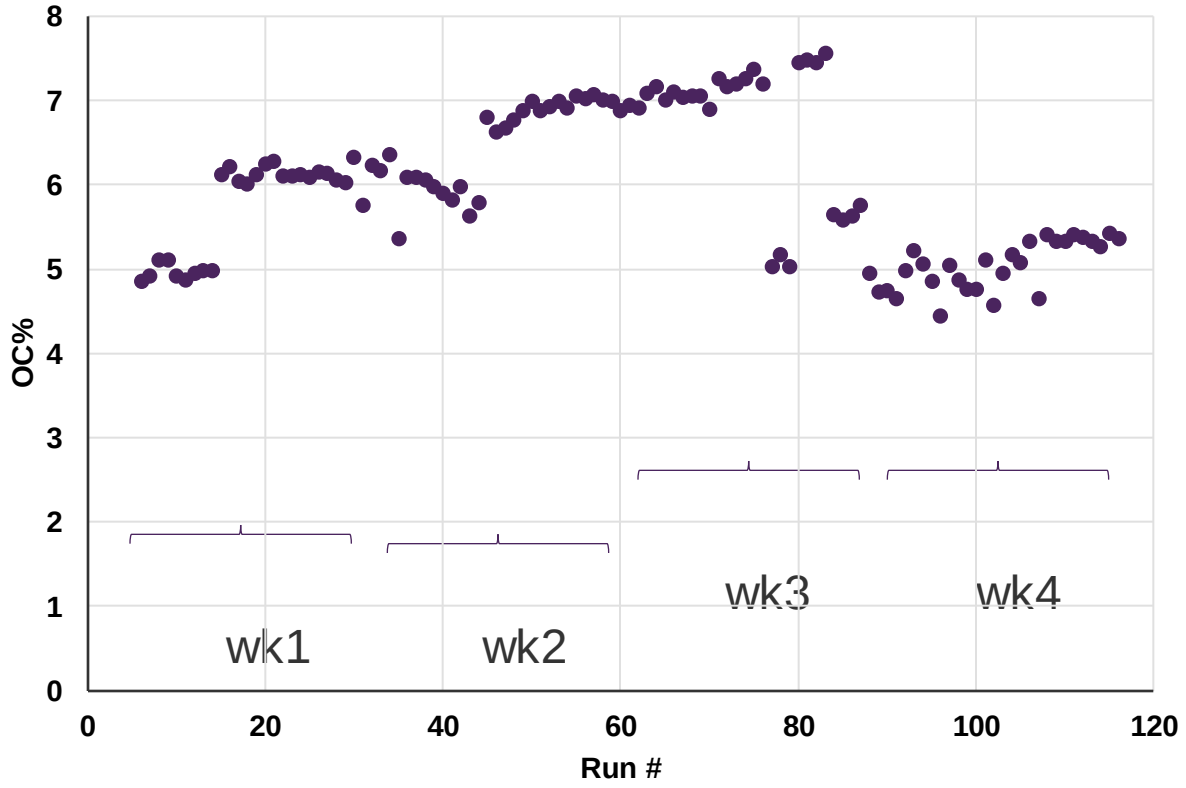


Long Term Precision of SC% and OC %

RSD: 2.2%

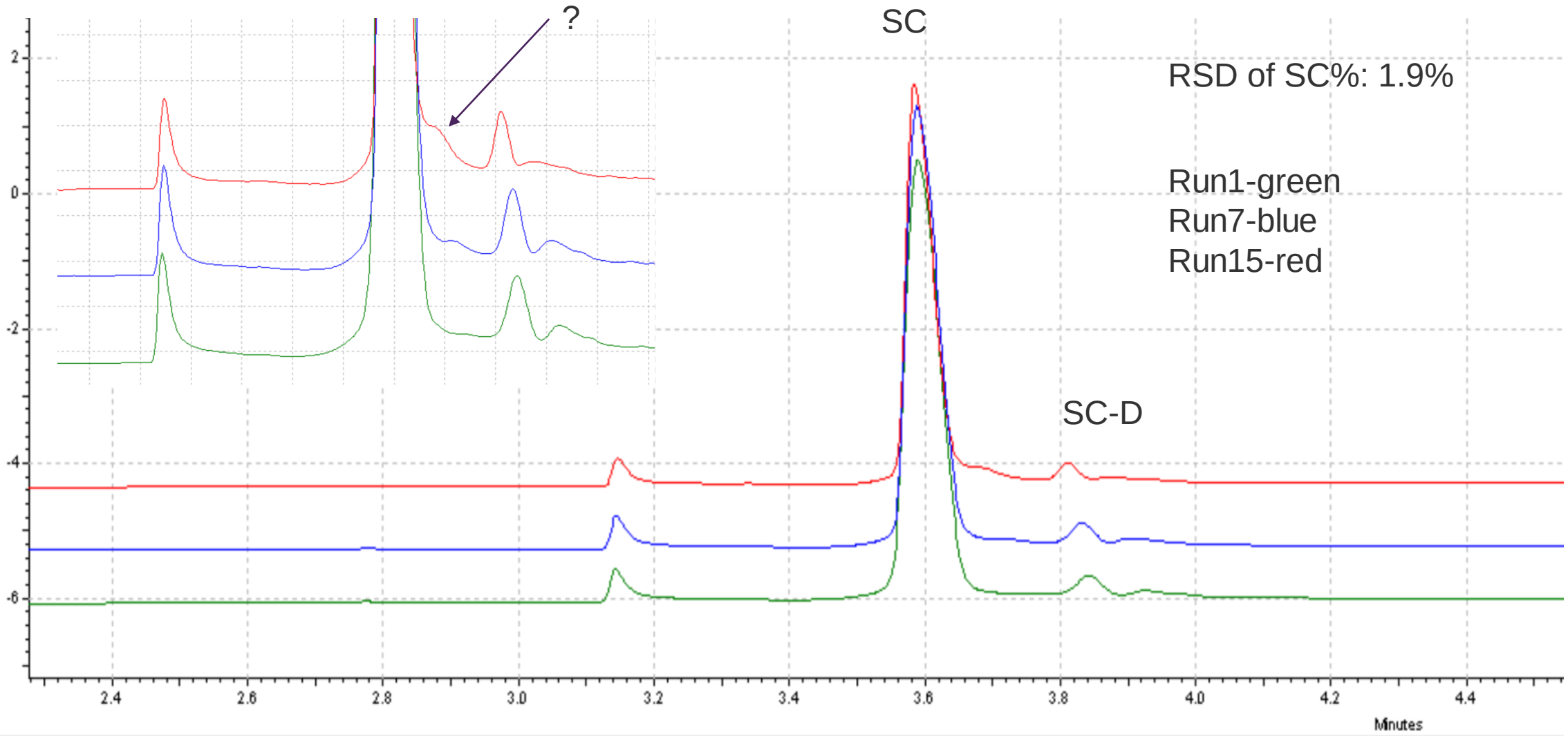


RSD1: 14% (total), RSD2: 5% (fresh sample)



Wk1-3: -20 C sample; Wk3-4: fresh sample

Short Term Precision-Intraday Reproducibility



Summary

1. CZE-UV with an uncoated capillary is a simple and cheap approach for fast and reproducible analysis of mAb charge variants
2. CGE-LIF with an uncoated capillary provide adequate long term precision in analysis of plasmid topoisomers. Short term precision needs to improved.
3. Capillary storage is important for long term reproducibility and capillary lifetime
4. Capillary lifetime is mAb or plasmid specific. More work will be done on the evaluation

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