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Improving ADC Sample Stability in iCIEF by Altering Separation Matrix pH – A Case Study

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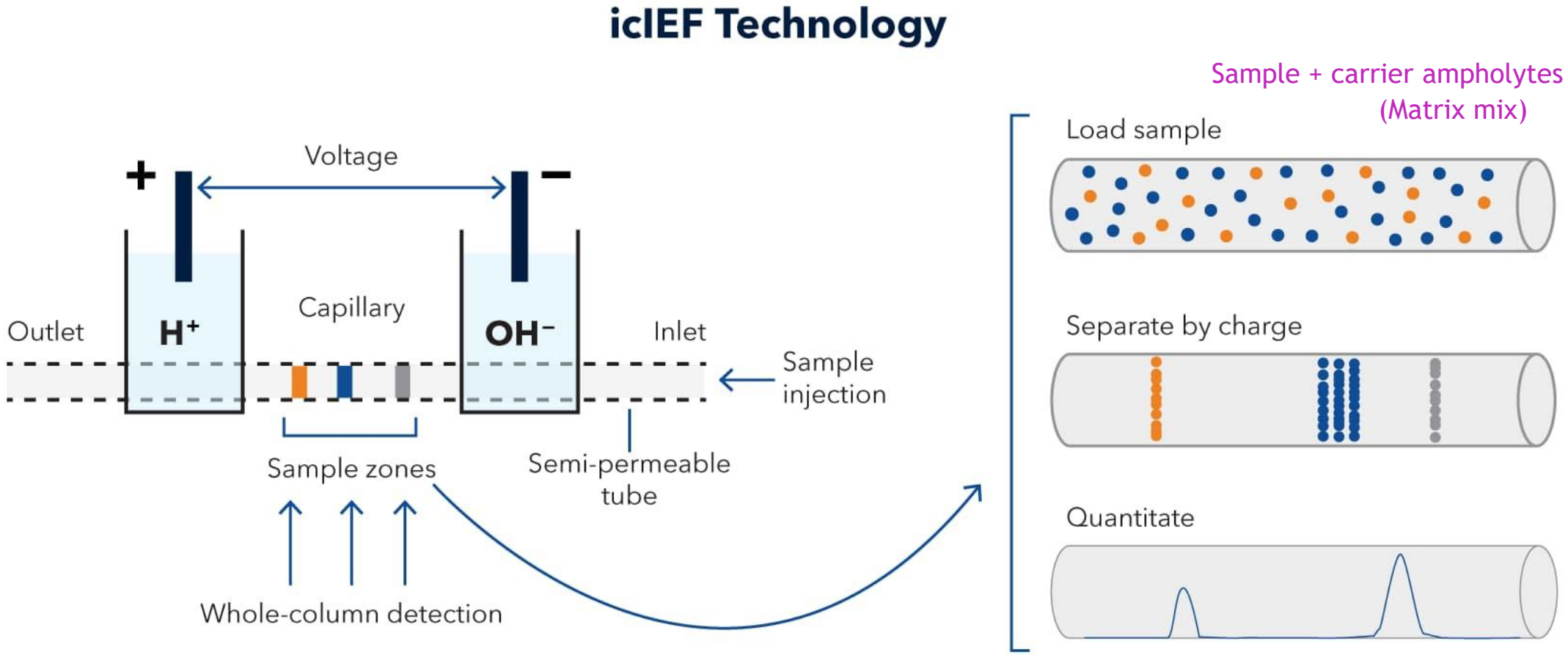
Outline

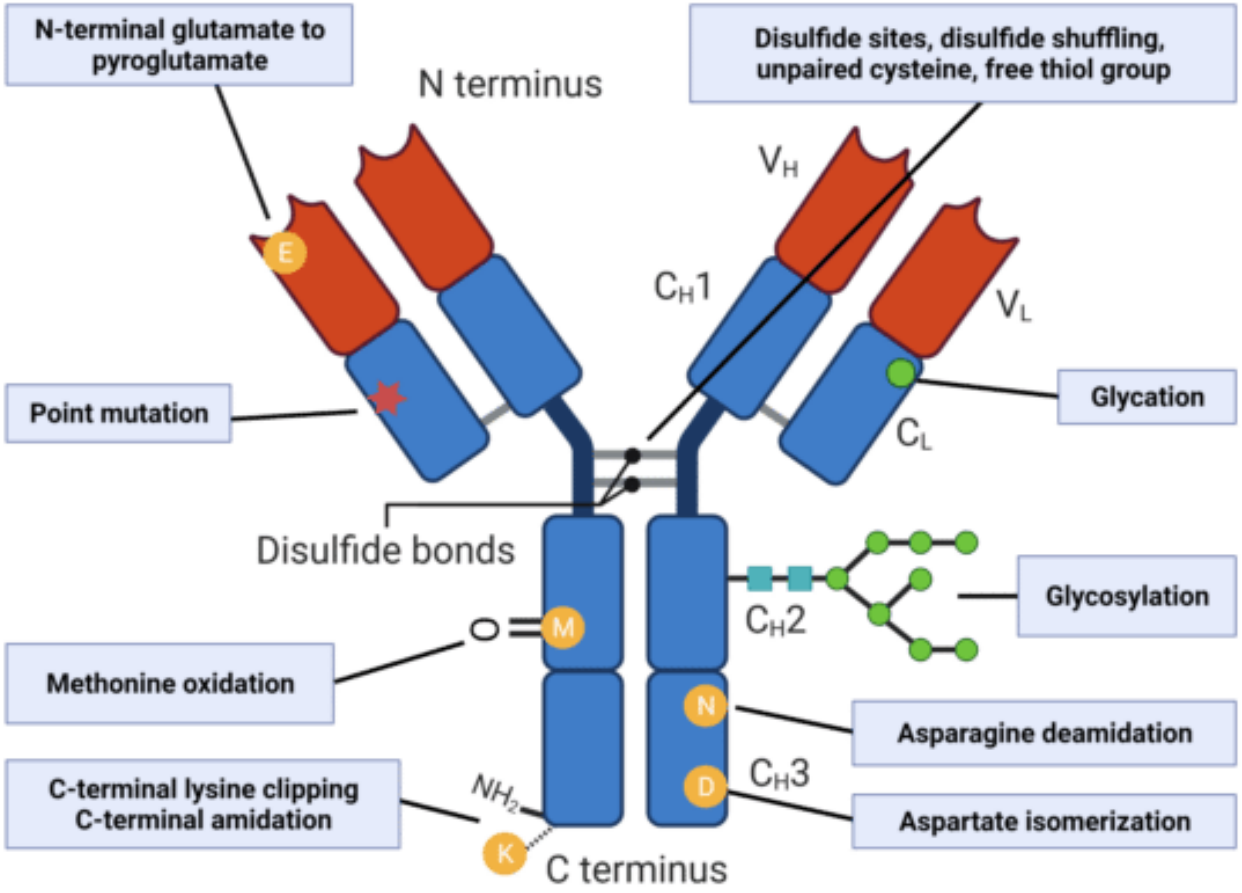
- Background
- The impact of the chemistry on charge variant analysis
- Analytical Strategies taken
- Case study

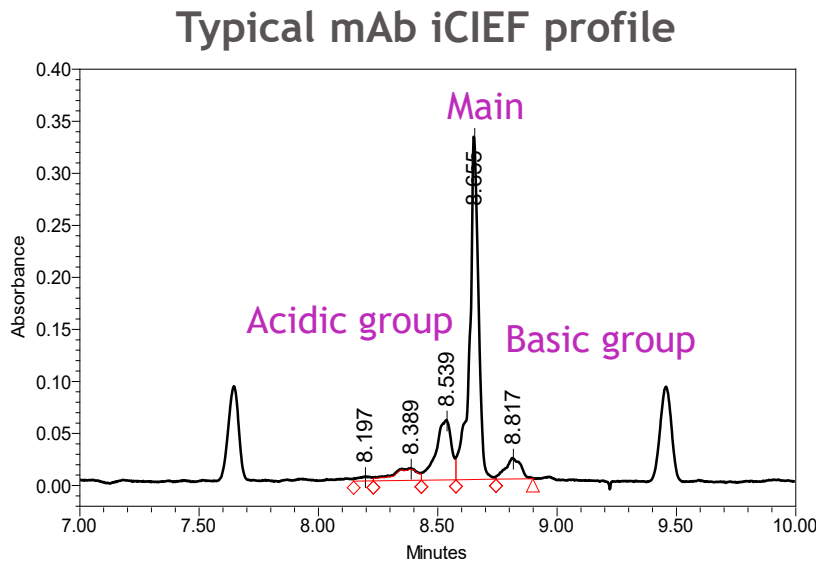
Imaged Capillary Isoelectric Focusing (iCIEF) Technology



https://www.bio-techne.com/resources/instrument-applications/protein-charge-heterogeneity-maurice-ice3

Post-translational Modifications and Charge Variants



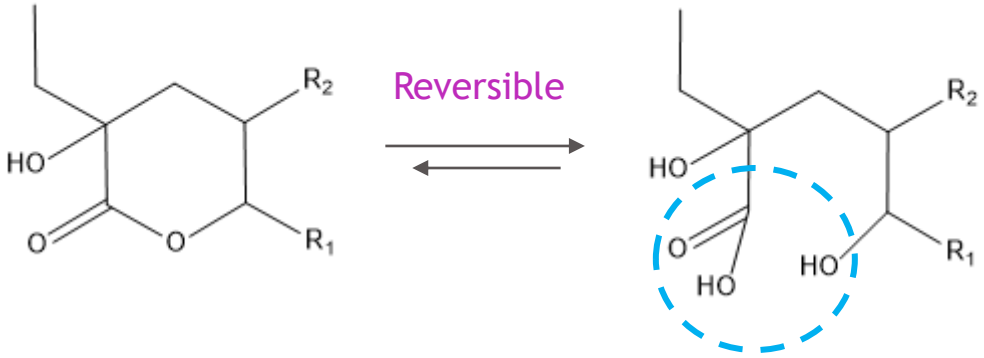


http://www.rapidnovor.com/why-do-post-translational-modifications-matter/

Chemistry: ADC structure and pH dependent hydrolysis

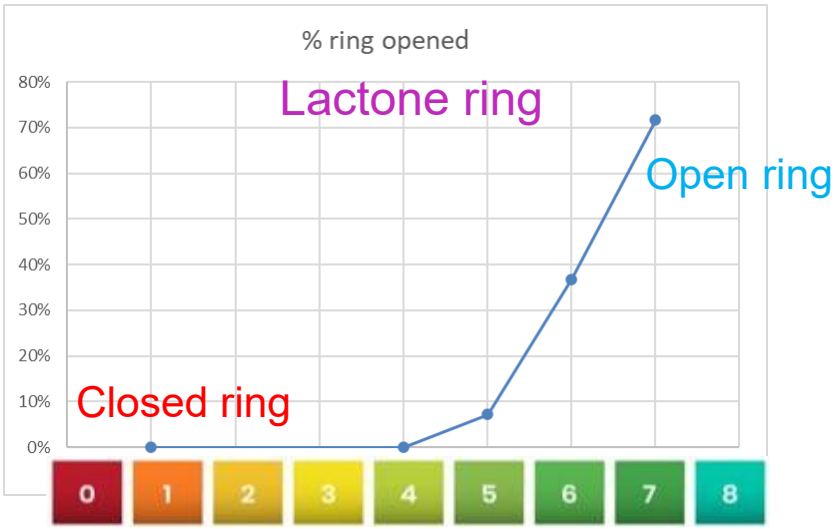
Antibody drug conjugates (ADC)

pH dependent equilibrium between lactone closed and open forms.



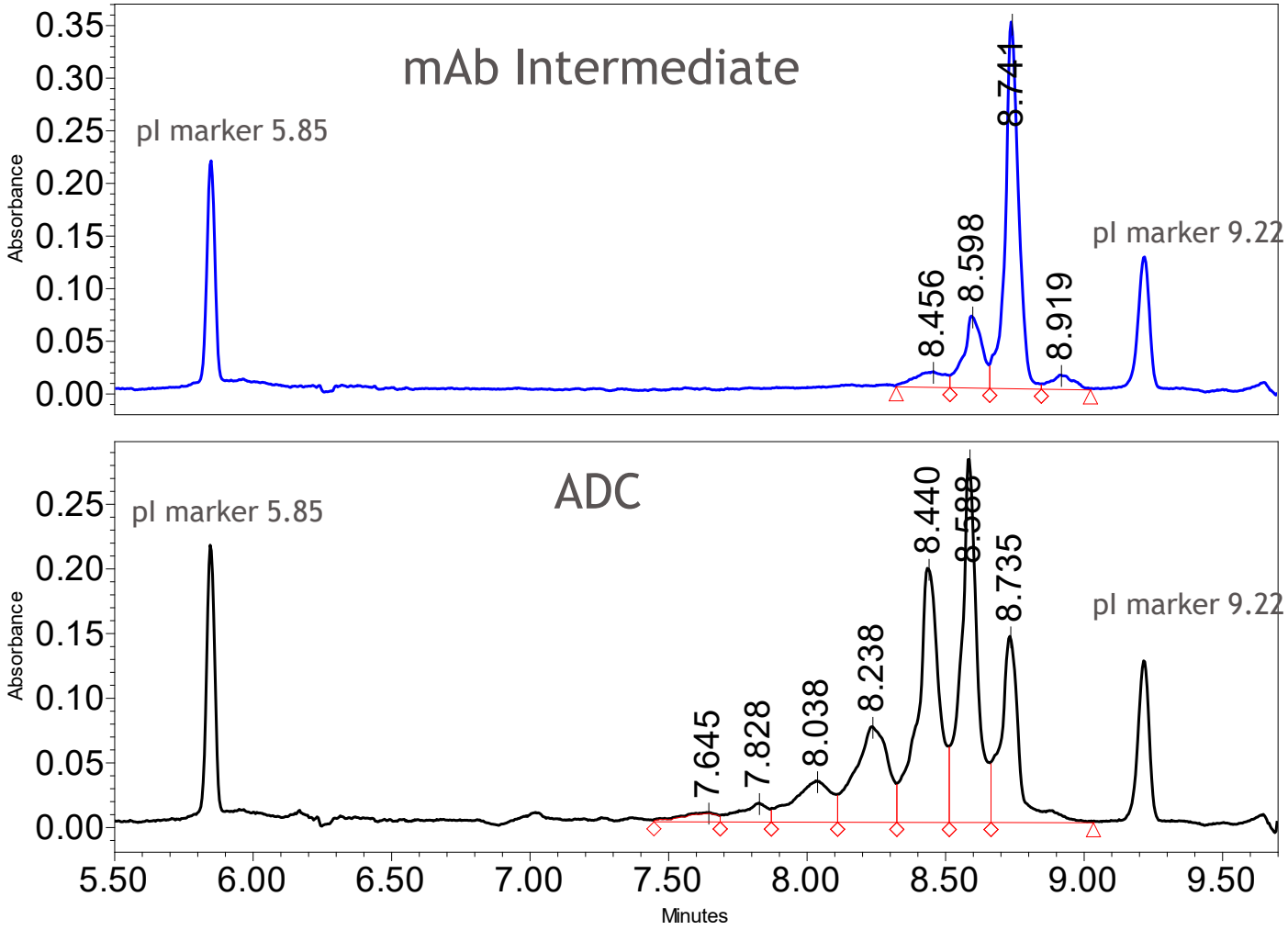
Closed ring

Open ring



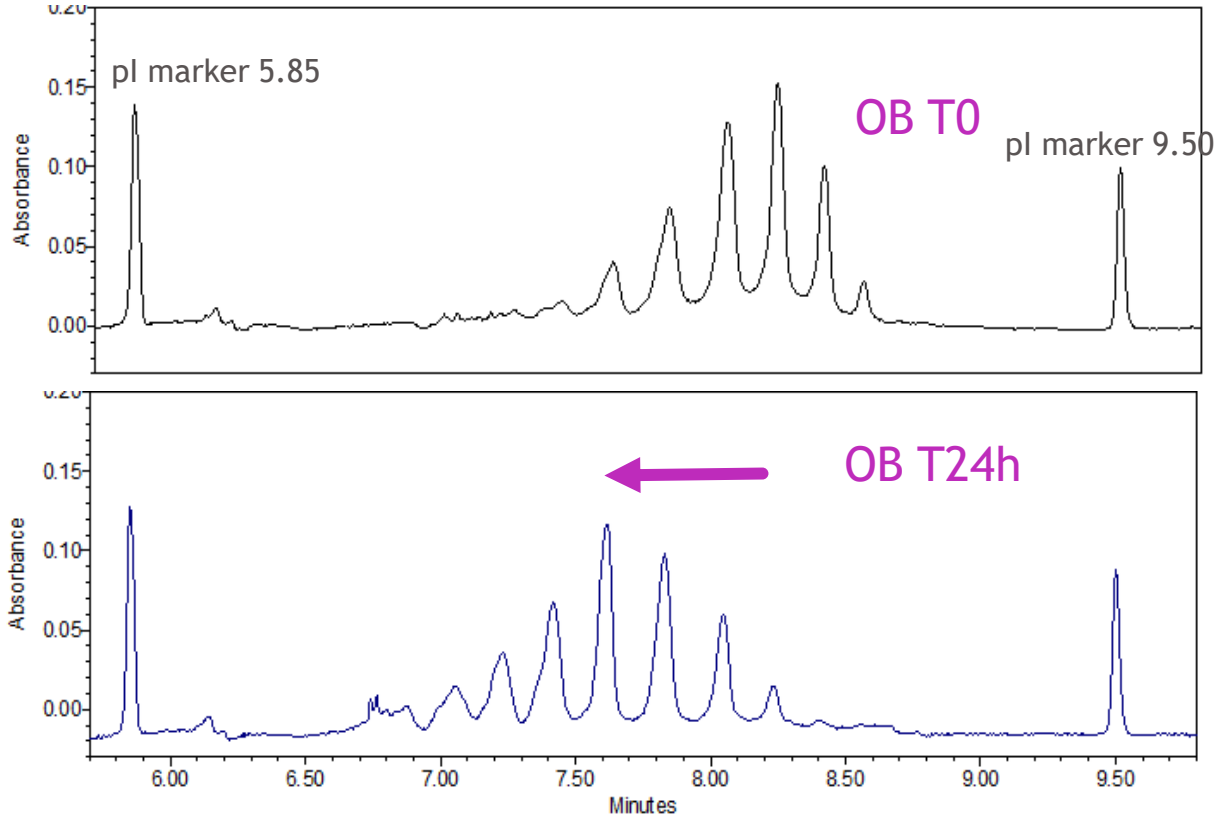
- Payload hydrolysis results in open ring form.
- This hydrolysis is pH dependent and reversible.
- Open ring forms can be reflected in iCIEF profile.

Challenges: ADC iCIEF Profile



- Multiple peaks
- Relative lower pI than mAb intermediate

Challenges: ADC iCIEF Profile On-Board (OB) Stability Issue



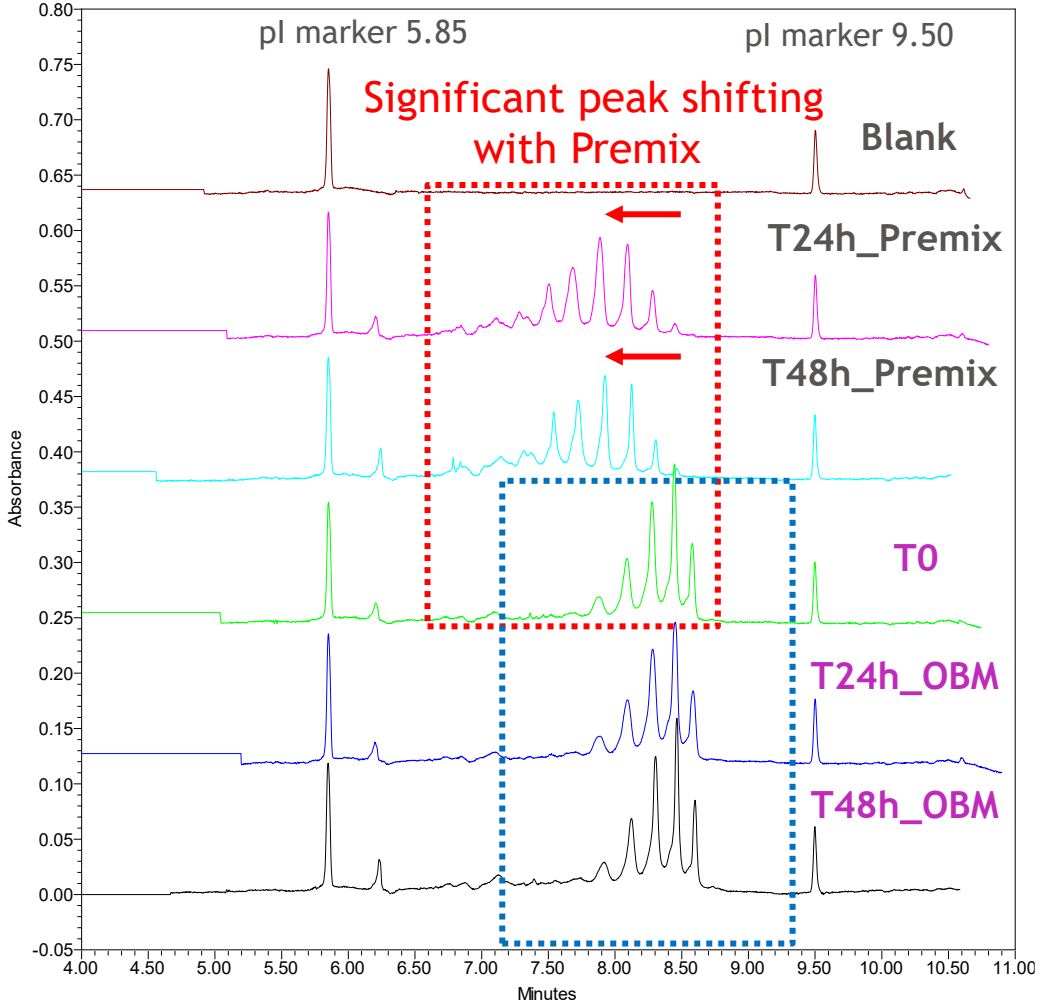
iCIEF method condition

Component	Matrix mix
Ampholyte(s)	2% Pharmalyte 3-10 2% Pharmalyte 8-10.5
Final Methyl Cellulose (MC) Concentration	0.35% MC
Protein Solubilizer	2M Urea
pI Markers: Low / High	5.85 / 9.50

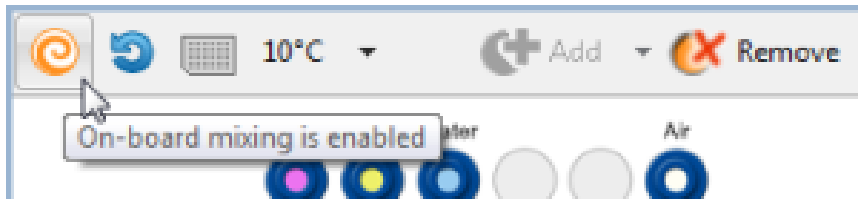
Matrix mix pH ~8
Formulation buffer pH ~6

- Over time acidic species increased.
- Payload hydrolysis caused iCIEF profile shifting when sample stored in autosampler (10ºC) and led to failures of system suitability.

Strategy 1: Reduce Master Mix and Sample Contact Time



Robust OB stability with OBM



Maurice

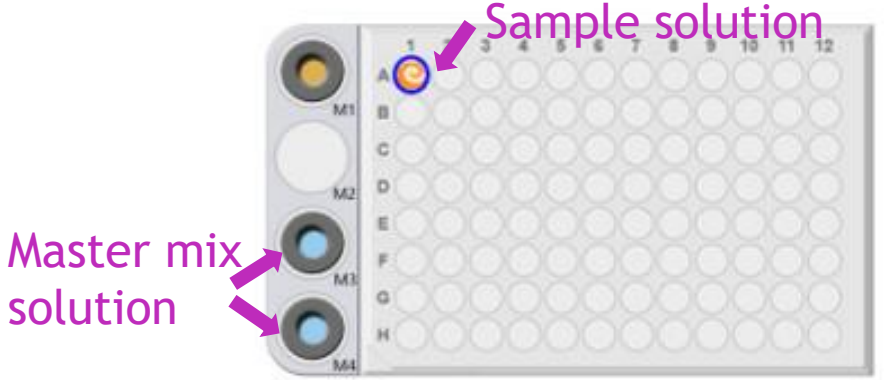
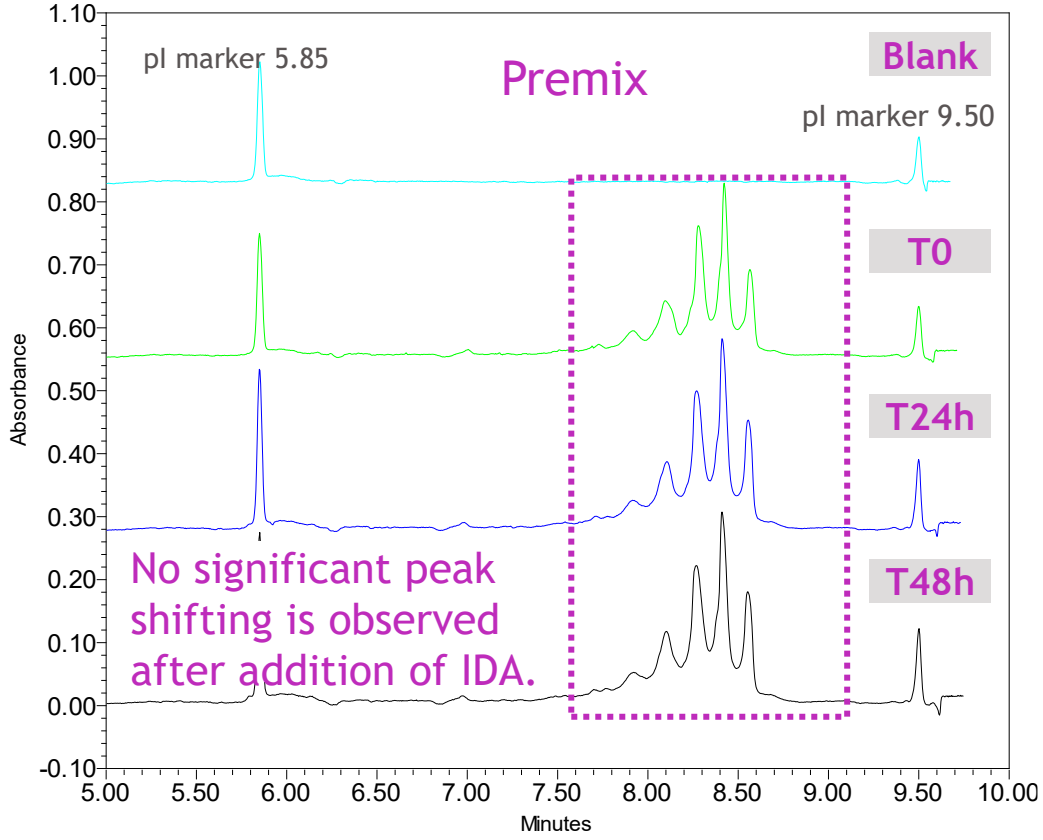


FIGURE 1. Reagent vial placement when setting up a Maurice cIEF batch with on-board mixing.

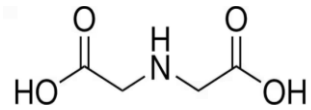
- ✓ With on-board mixing (OBM), the master mix was added into each sample immediately prior to iCIEF injection to minimize their contact time.
- ✓ This approach met both system suitability criteria and on-board stability requirements.

Strategy 2: Could Lower Master Mix pH Improve OB Stability?



Component	Matrix Mix
Ampholyte(s)	2% Pharmalyte 3-10, 2% Pharmalyte 8-10.5
Final Methyl Cellulose (MC) Concentration	0.35% MC
Protein Solubilizer	SimpleSol
pI Markers: Low / High	5.85 / 9.50
Additives	25 mM IDA

Matrix mix pH ~6



Iminodiacetic acid (IDA)

- With addition of iminodiacetic acid in master mix, DS sample showed good on-board stability in autosampler (10°C) for up to 48 hours.

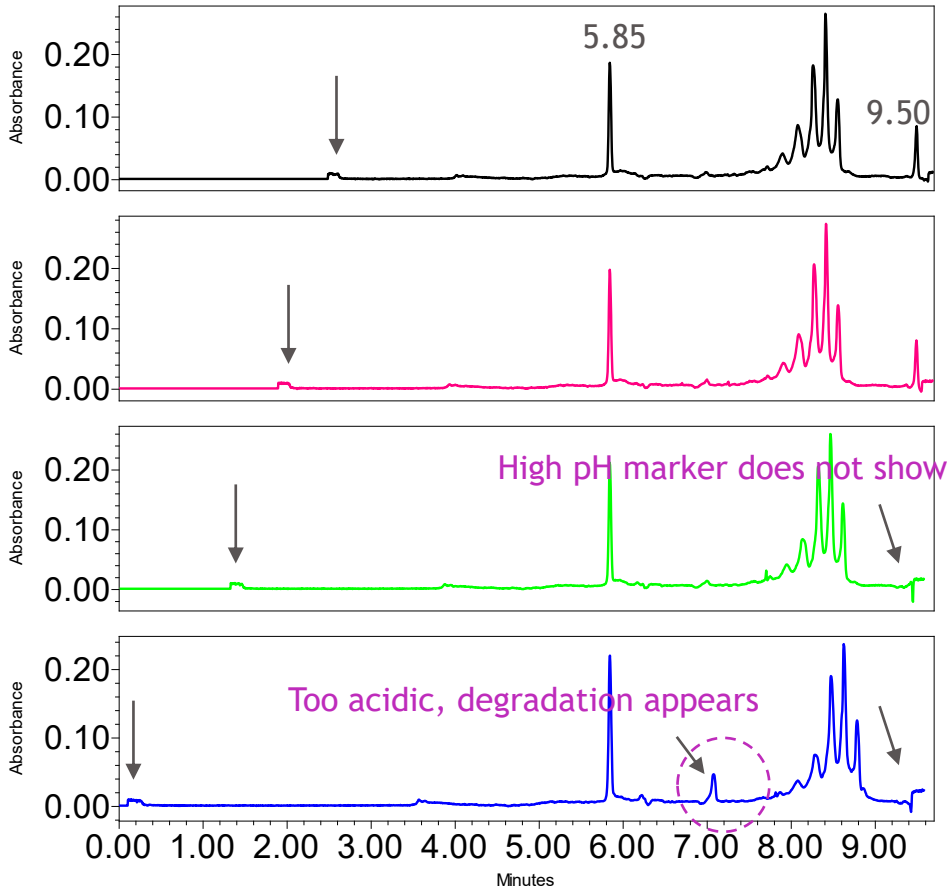
Method Development
IDA conc. – Wide range study (Screening)

IDA final conc. 20mM
pH 6.4

IDA final conc. 25mM
pH 6.0

IDA final conc. 30mM
pH 5.6

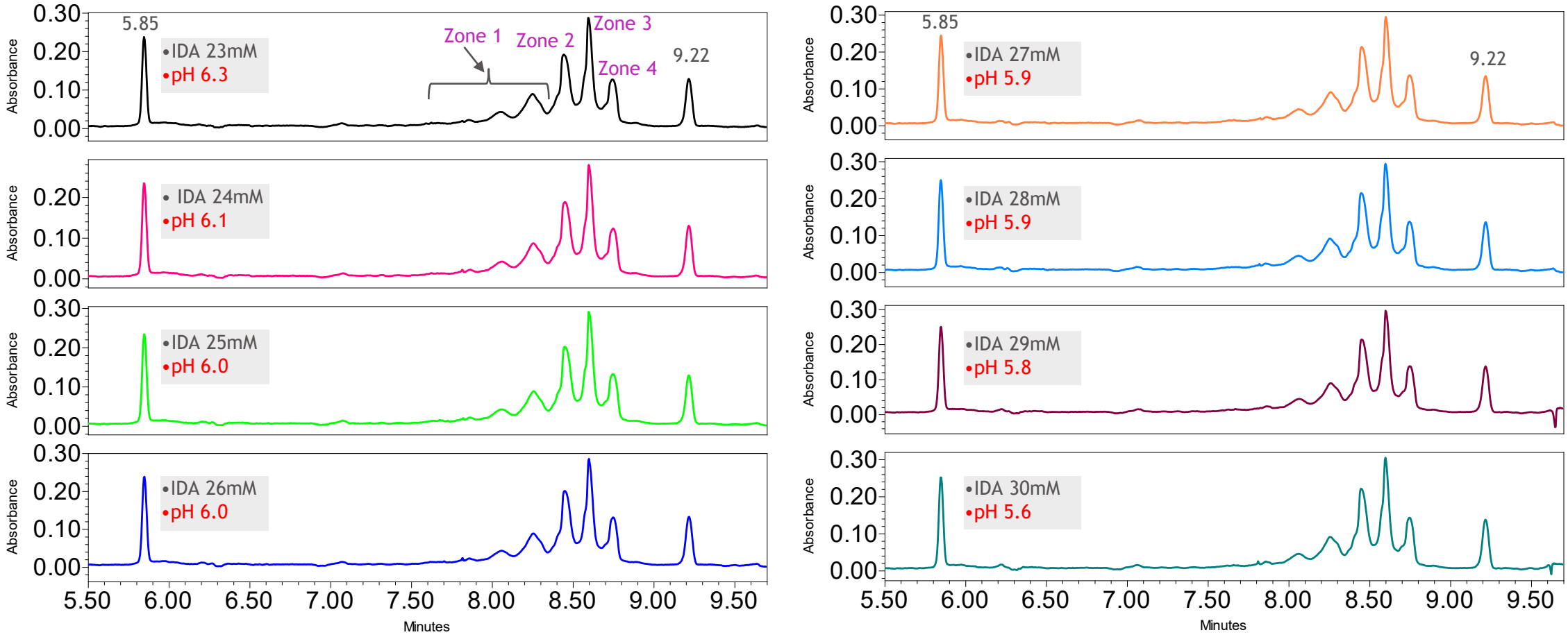
IDA final conc. 35mM
pH 4.9



IDA is an acidic spacer, which shifts all peaks towards the right end.

- If more than 35 mM IDA is added to master mix, it would cause degradation and high pI marker may not show.

IDA conc. – Narrow Range Study (Robustness)



- Within 23 mM to 30 mM IDA range, the DS samples showed similar iCIEF profiles.

IDA conc. – Narrow Range Study (Robustness) cont'd

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 23mM pH 6.3	T0	30.0	25.9	28.2	15.9
	T24h	32.6	26.9	26.3	14.2
	T48h	33.7	26.8	26.8	13.6

Change from initial ≥ 2.5

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 24mM pH 6.1	T0	30.2	26.3	27.9	15.6
	T24h	32.2	26.5	26.9	14.4
	T48h	33.0	26.3	26.5	14.2

Change from initial ≥ 2.5

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 25mM pH 6.0	T0	29.1	26.5	28.1	16.2
	T24h	30.7	26.9	27.3	15.1
	T48h	31.2	27.1	26.6	15.1

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 26mM pH 6.0	T0	29.9	26.5	27.9	15.7
	T24h	30.6	27.0	27.0	15.4
	T48h	31.1	26.9	27.0	15.0

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 27mM pH 5.9	T0	30.0	27.3	27.1	15.6
	T24h	30.0	27.3	27.1	15.6
	T48h	30.2	27.1	27.1	15.6

	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 28mM pH 5.9	T0	30.0	27.0	27.6	15.4
	T24h	30.1	27.3	27.1	15.6
	T48h	30.3	27.4	26.7	15.6

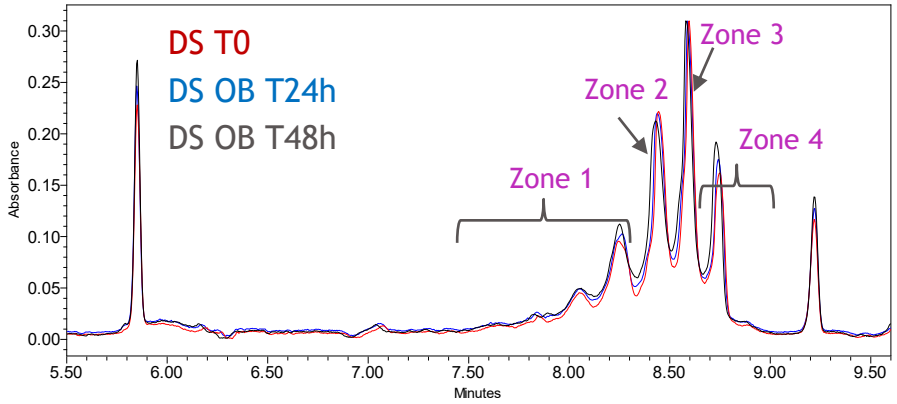
	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 29mM pH 5.8	T0	29.5	26.9	28.1	15.5
	T24h	29.4	27.4	27.4	15.8
	T48h	29.4	27.7	26.7	16.2

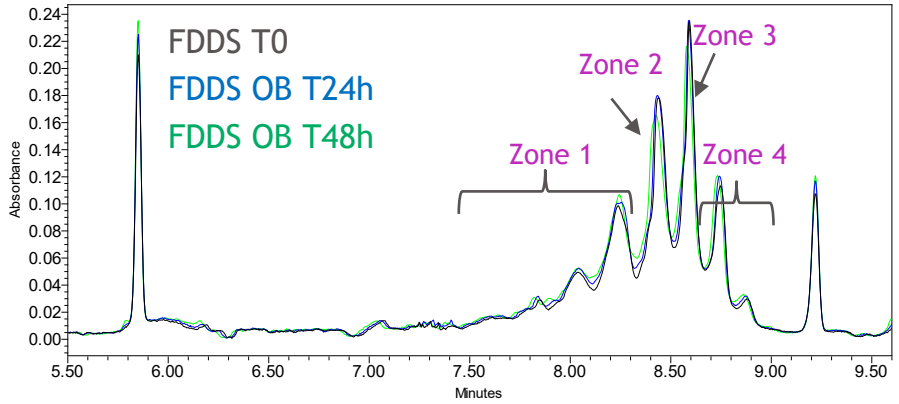
	Sample	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
IDA 30mM pH 5.6	T0	29.3	27.2	27.8	15.7
	T24h	29.1	27.5	27.2	16.2
	T48h	29.0	27.6	27.0	16.4

- Good on-board stability is shown when IDA is in 28±2 mM concentration range.

(Considering worst case sample prep variation for IDA preparation (5% weight variation and 3% pipetting variation), it will lead to +/- 2 mM IDA concentration change.)

DS and FDDS On-board Stability with Final Method Conditions





Component	Method condition
Instrument	Maurice
Ampholyte(s)	Pharmalyte 3-10 Pharmalyte 8-10.5
Final Methyl Cellulose (MC) Concentration	0.35% MC
Protein Solubilizer	SimpleSol
pI Markers: Low / High	5.85 / 9.22
Additives	28 mM IDA
Autosampler Temperature	10 °C
Detection	UV (280 nm)

Injection	Time (Hrs.)	A% Zone 1	A% Zone 2	A% Zone 3	A% Zone 4
DS	Initial (0)	29.0	28.0	26.9	16.1
DS	24	28.8	27.5	27.5	16.2
DS	48	29.2	26.9	28.4	15.5
FDDS	Initial (0)	34.7	26.1	23.5	15.8
FDDS	24	34.6	26.4	23.1	15.9
FDDS	48	35.4	25.6	23.9	15.0

- ✓ System suitability
- ✓ Precision
- ✓ Accuracy
- ✓ Specificity
- ✓ Linearity
- ✓ On-board stability

Conclusion

- ADC contains payload with lactone ring, which is liable to reversible and pH dependent hydrolysis.
- The hydrolysis could cause iCIEF profile shifting and lead to failures in system suitability and on-board stability.
- To enhance the on-board stability of ADC samples, two strategies have been employed at BMS: (1) on-board mixing to minimize the contact time between sample and separation matrix solution, and (2) adjusting the separation matrix to a lower pH to improve sample stability.

Acknowledgement

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