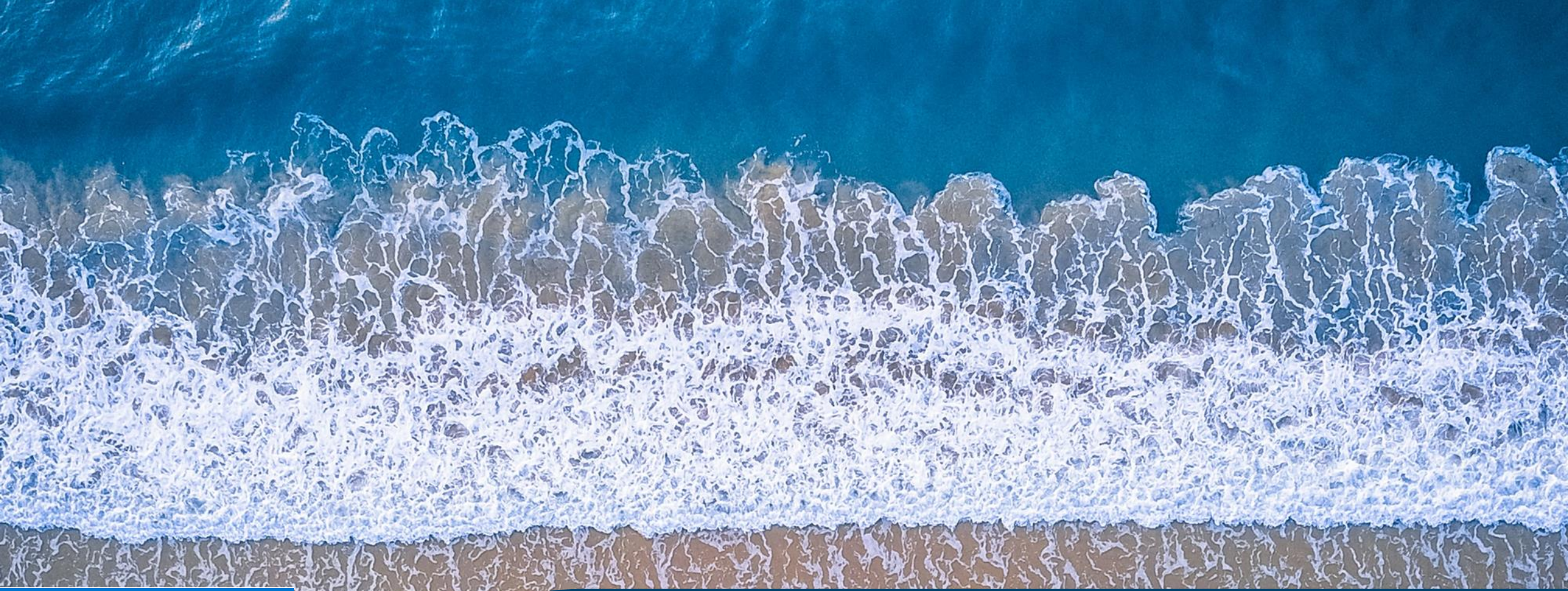


Where science
& creativity meet

INTRODUCTION TO IFF

Keaton Albers– Senior Applications Associate II

IFF - APPLIED INNOVATION CENTER

Cedar Rapids, Iowa

Lab-based plant support

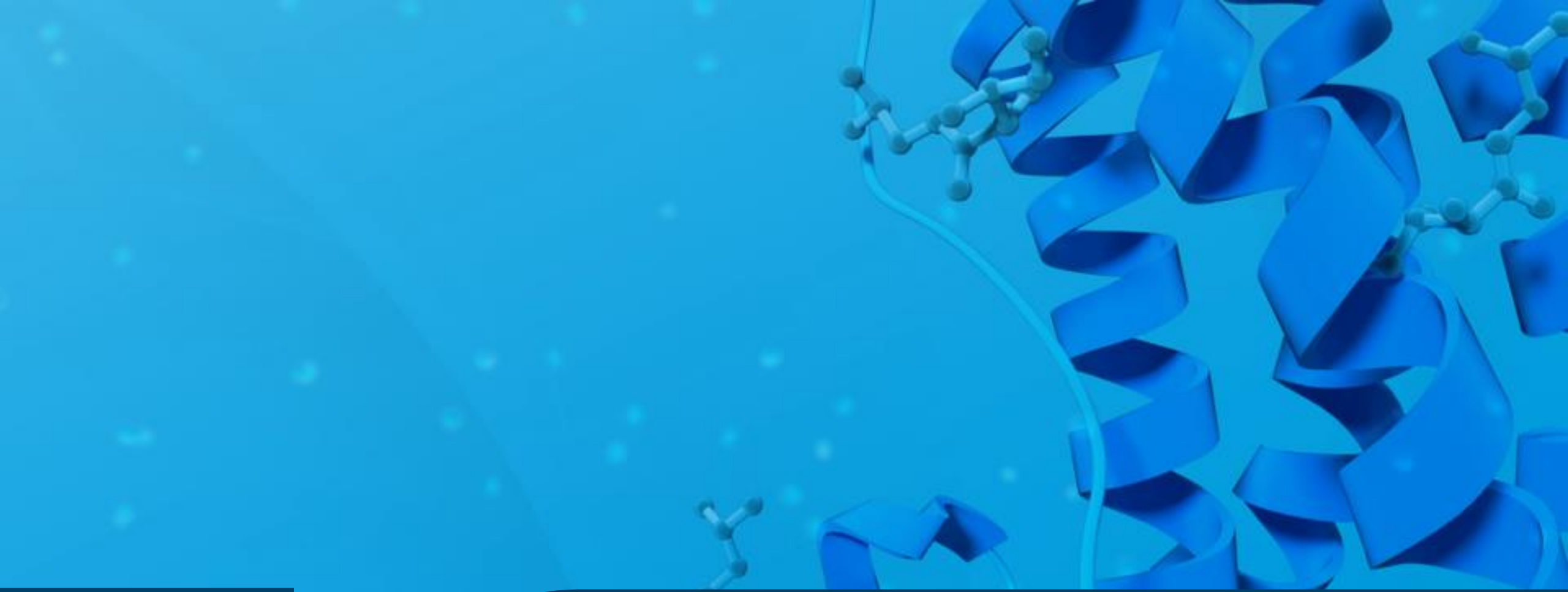
- Pre-trial testing
- Trial evaluation
- Optimization
- Troubleshooting

Liquefaction Services

- Cook studies
- Solubility
- Cations (Sodium, etc.)

Fermentation Services

- Prop and ferm studies
- DP4+ composition
- Detailed sugar analysis
- HPLC checks
- Residual starch
- Nitrogen measurements
- Inhibitors (fusels, sodium, sulfite, organic acids, etc.)



XCELIS® Ethanol Solutions

HPLC TROUBLESHOOTING AND TRAINING

HPLC TROUBLESHOOTING

Diagnostic evaluation

Two main types of problems

Hardware issues

- Column or other consumables
- Major system error

Software issues

- Integration or calibration issues

How is this different from normal operations?

- Keep records of normal operations (or be able to find previous data)
- Run check standards

Start with hardware problems

- Quick to check

HARDWARE PROBLEMS

Changes in physical parameters usually indicate a hardware issue

Quick and easy-to-spot changes

- Initial check can be done in <5 minutes with good record keeping

What changes/observations are the operators/lab manager seeing when the HPLC is operating?

How is this different from normal operations?

Keep records of normal operations (or be able to find previous data)

- Pressure
- Retention time
- Peak shape (especially width and tailing)
- Area (choose a Standard)

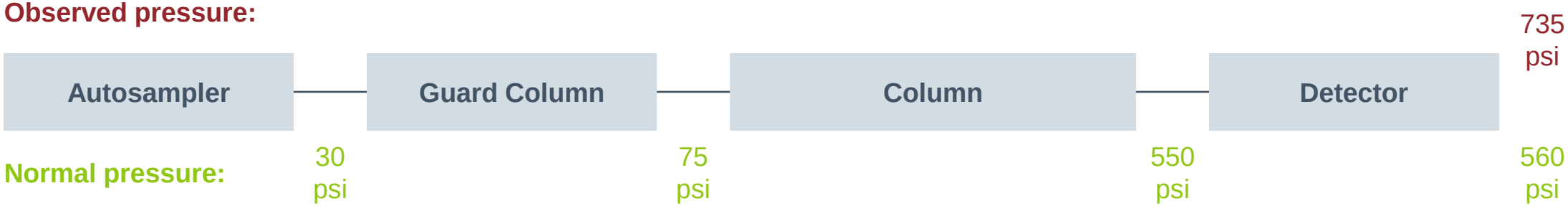
HARDWARE PROBLEMS

High pressure

First step: Find the pressure source

- Start from detector, work backward, and remove components until pressure source is found
- Example 1:

Observed pressure:



Replace plugged part

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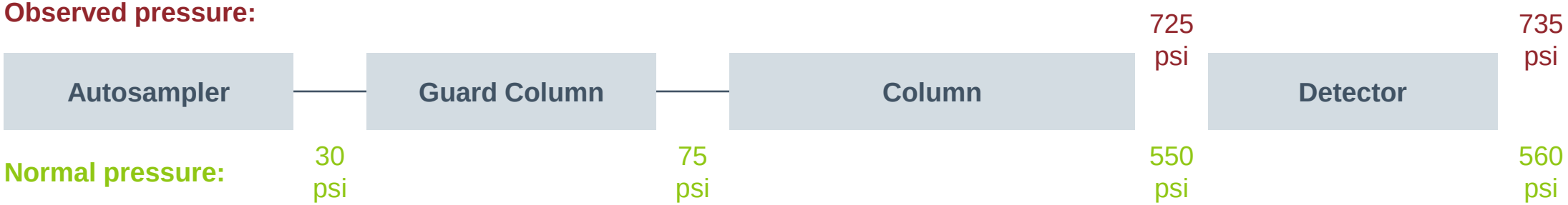

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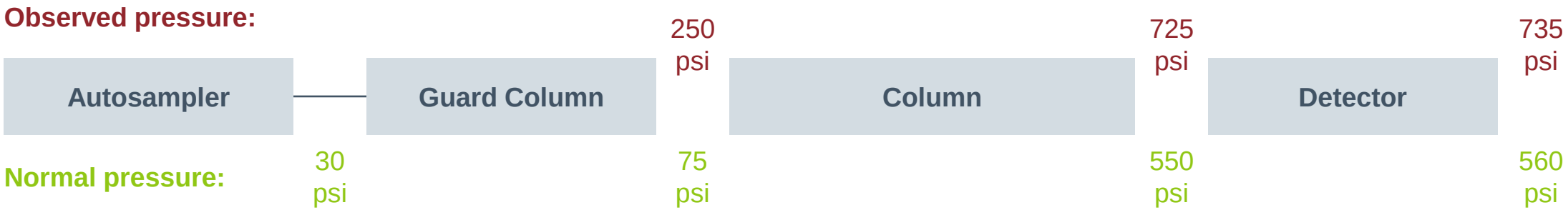

Replace plugged part

HARDWARE PROBLEMS

High pressure

First step: Find the pressure source

- Start from detector, work backward, and remove components until pressure source is found
- Example 1:

Observed pressure:	30 psi	**Autosampler**	250 psi	**Guard Column**	725 psi	**Column**	735 psi	**Detector**
Normal pressure:	30 psi		75 psi		550 psi		560 psi	

Replace plugged part

HARDWARE PROBLEMS

High pressure

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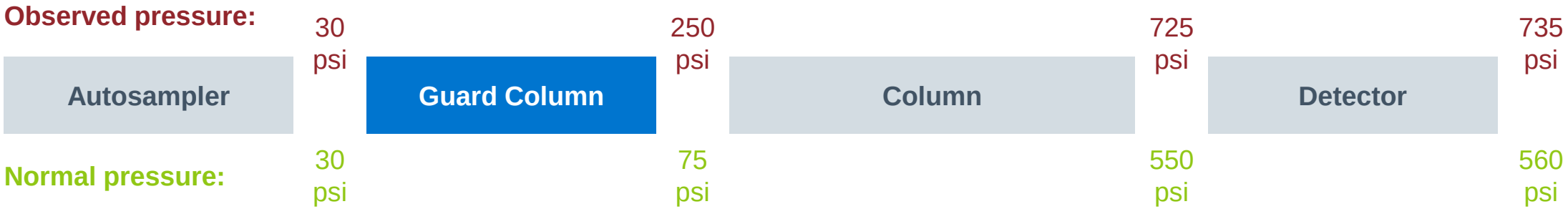
- Start from detector, work backward, and remove components until pressure source is found
- Example 1:



Replace plugged part

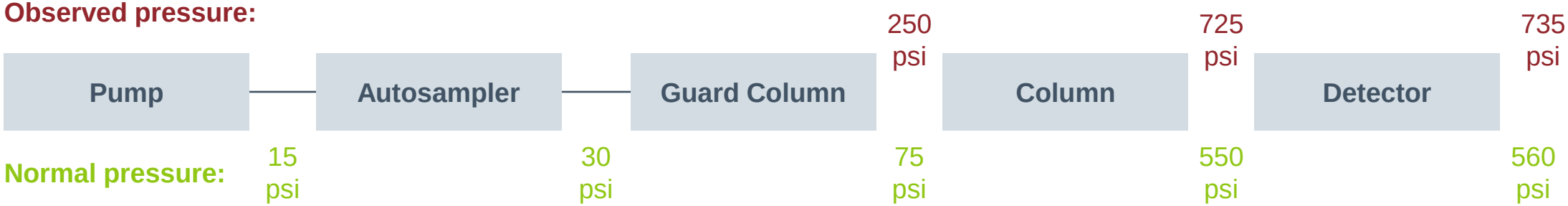
HARDWARE PROBLEMS

High pressure

First step: Find the pressure source

- Start from detector, work backward, and remove components until pressure source is found
- Example 2:

Observed pressure:



Replace plugged part

HARDWARE PROBLEMS

High pressure

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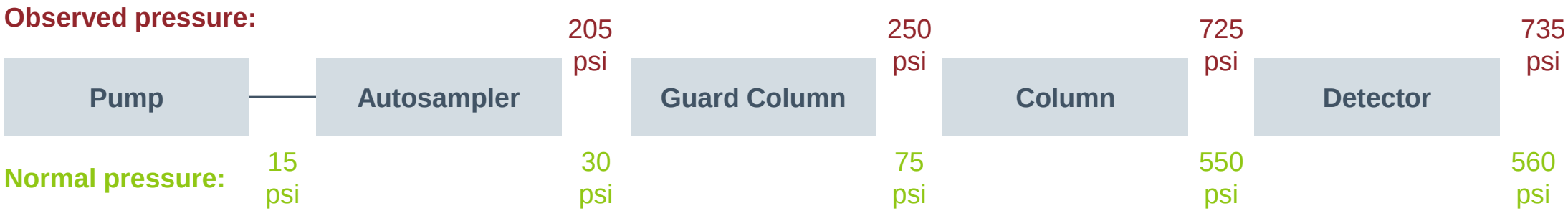

Replace plugged part

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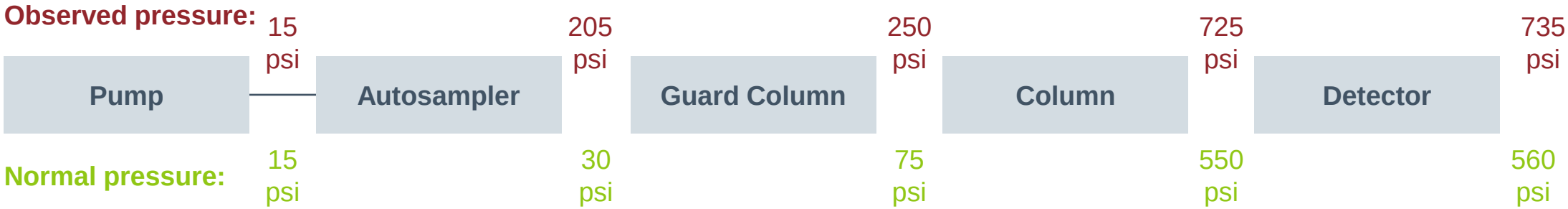

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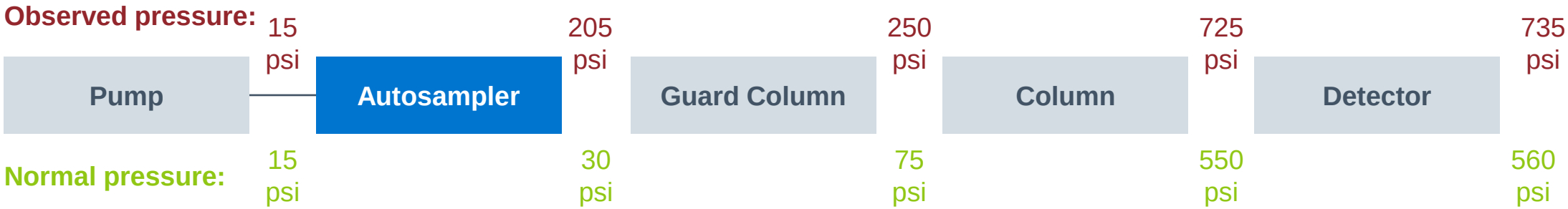


Replace plugged part

Public

HARDWARE PROBLEMS

Old Column

Retention time:

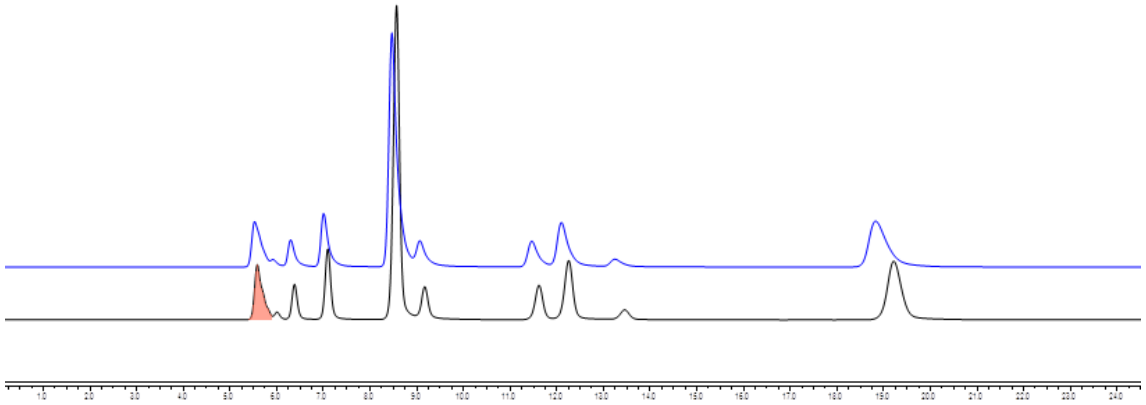
Compare retention times to previous runs

- Standards work best
- If retention times changed, check peak windows and peak IDs

Peak shape

- Wide peaks and tailing peaks both usually indicate column issues
- Often only solution is to replace column
- Can sometimes indicate old tubing or pump issues

Blue column needs replaced



HARDWARE PROBLEMS

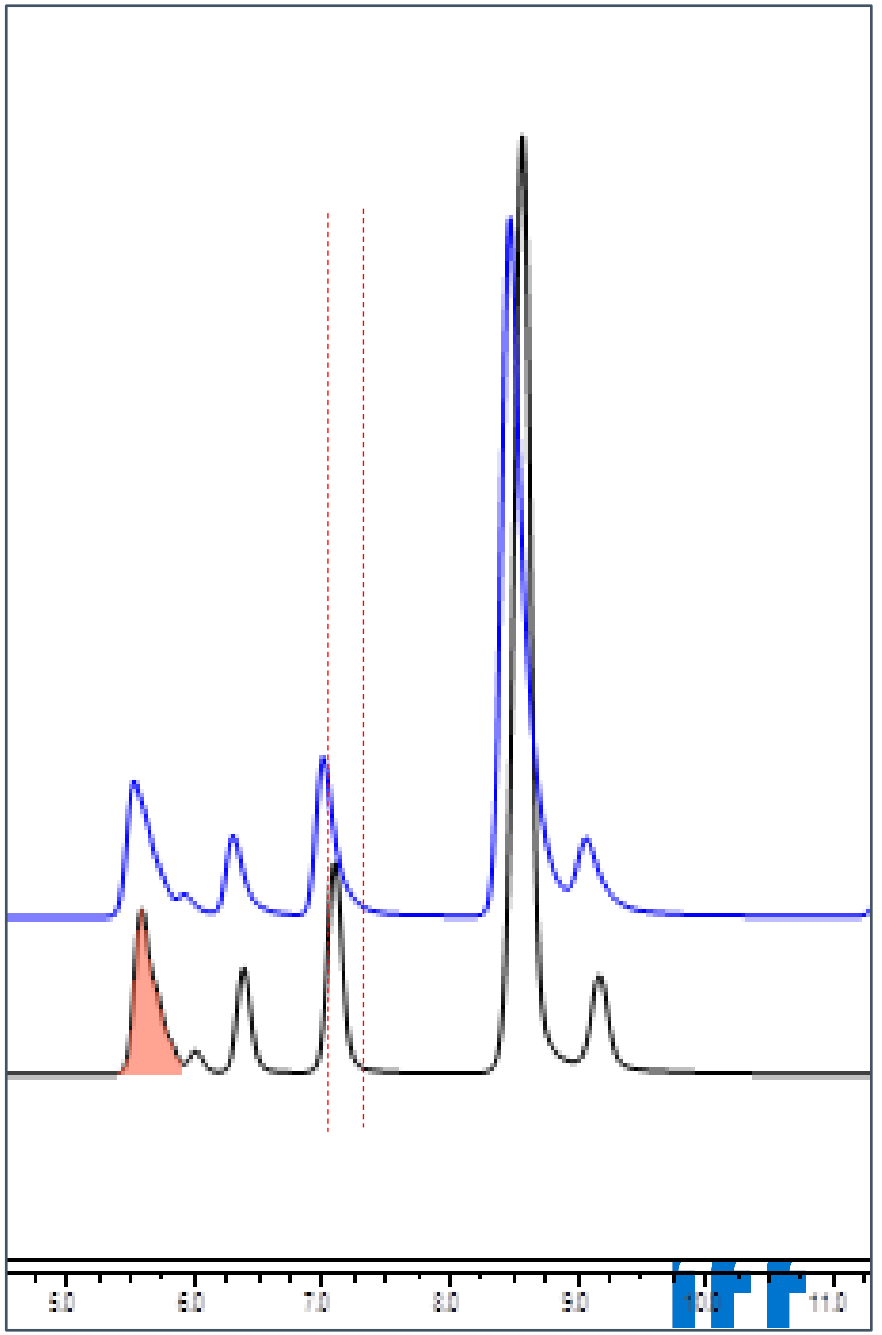
Changing Columns

Retention Time Changes

Peak IDs can change as small retention time changes can move peaks outside of peak ID window

Coelution changes

Peak splitting, especially around DP4+, DP2, DP1



HARDWARE PROBLEMS

Changing Columns

Retention Time Changes

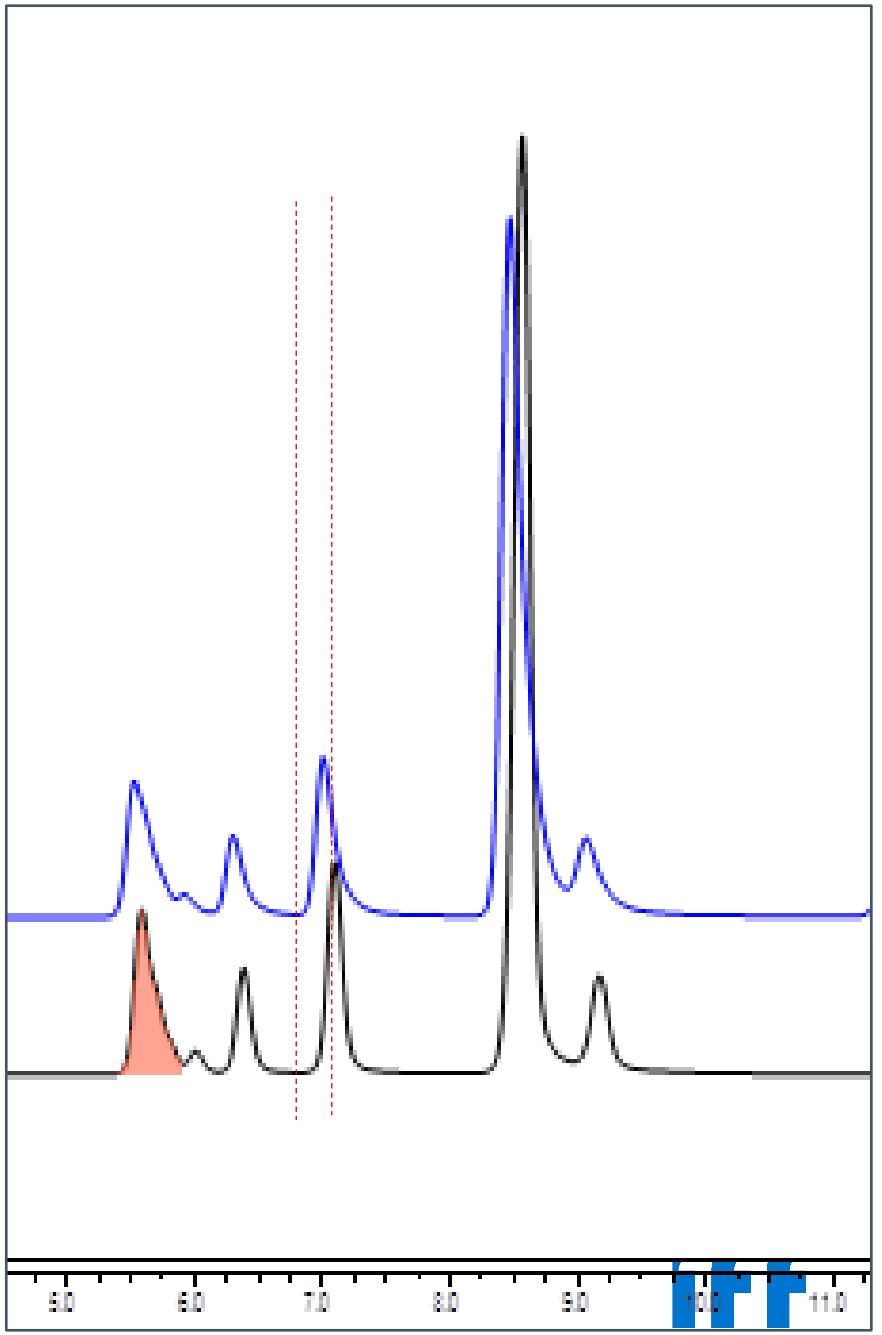
Peak IDs can change as small retention time changes can move peaks outside of peak ID window

Coelution changes

Peak splitting, especially around DP4+, DP2, DP1

Improving column performance can cause problems

Same issues as poor performance, but in reverse



HARDWARE PROBLEMS

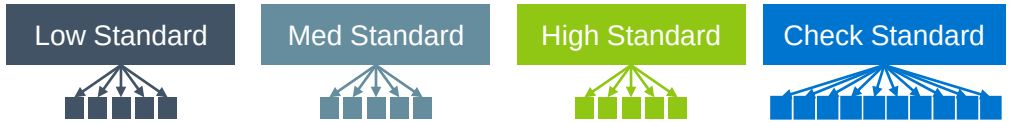
Inconsistent Areas

- **Standard area check**
- **Compare area of standard or check standards to previous runs of the same standard**
- **Change in area indicates a possible HPLC problem**
- **If no other issues are present (integration, peak shape, etc.), changes in area may indicate a problem with the standard, autosampler, or detector**

Date	Pressure	DP1 RT	DP1 Area	EtOH RT	EtOH Area
4/3	466	9.82	100563	20.1	100468
4/5	469	9.83	101571	19.8	99731
4/10	482	9.81	103648	19.9	99464
4/12	470	9.85	99615	19.9	100753
4/14	476	9.83	80640	20.0	82376

REPRODUCIBILITY CHECK

- **Make Standards and check standards and place in 5-10 vials**



- **Inject the vials once or twice per day as follows (vary time of day if once/day) on each HPLC :**

Day 1 AM	Low Standard 1	Med Standard 1	High Standard 1	Check Standard 1	Check Standard 1	Check Standard 1
Day 1 PM				Check Standard 2	Check Standard 2	Check Standard 2
Day 2 AM	Low Standard 2	Med Standard 2	High Standard 2	Check Standard 3	Check Standard 3	Check Standard 3
Day 2 PM				Check Standard 4	Check Standard 4	Check Standard 4
Day 3 AM	Low Standard 3	Med Standard 3	High Standard 3	Check Standard 5	Check Standard 5	Check Standard 5
Day 3 PM				Check Standard 6	Check Standard 6	Check Standard 6
Day 4 AM	Low Standard 4	Med Standard 4	High Standard 4	Check Standard 7	Check Standard 7	Check Standard 7
Day 4 PM				Check Standard 8	Check Standard 8	Check Standard 8
Day 5 AM	Low Standard 5	Med Standard 5	High Standard 5	Check Standard 9	Check Standard 9	Check Standard 9
Day 5 PM				Check Standard 10	Check Standard 10	Check Standard 10

- **Results (%wt/v) should be the same for each check standard run**
- **If results vary, HPLC system has a reproducibility problem**
- **This can prove that your system has an issue**

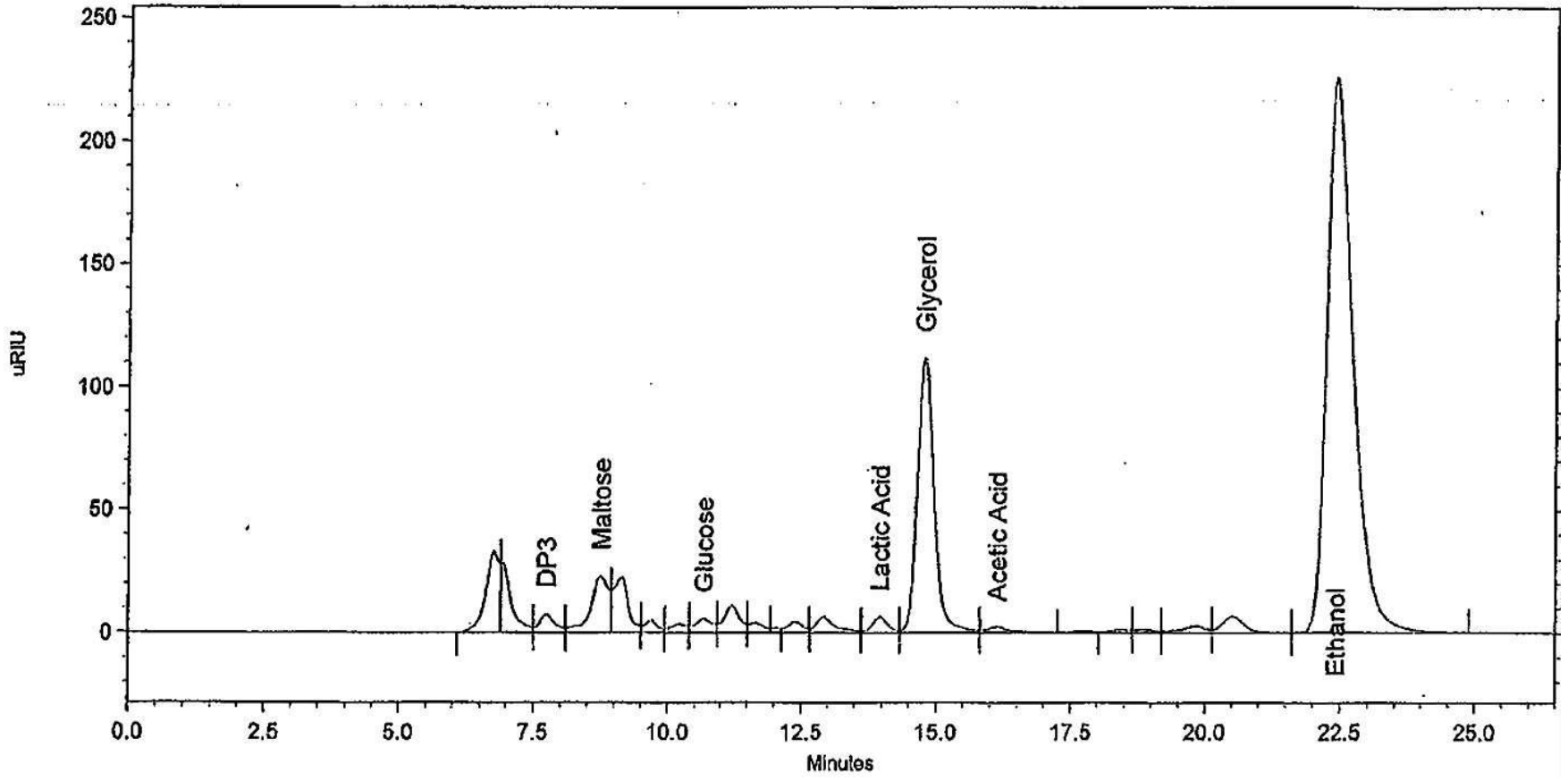
SOFTWARE PROBLEMS

If there is no obvious instrument malfunctions, check data analysis problems

Common data analysis problems

- Peak identifications
- Peak integrations
- Calibration errors

STANDARD CORN-TO-ETHANOL FERMENTATION HPLC CHROMATOGRAM



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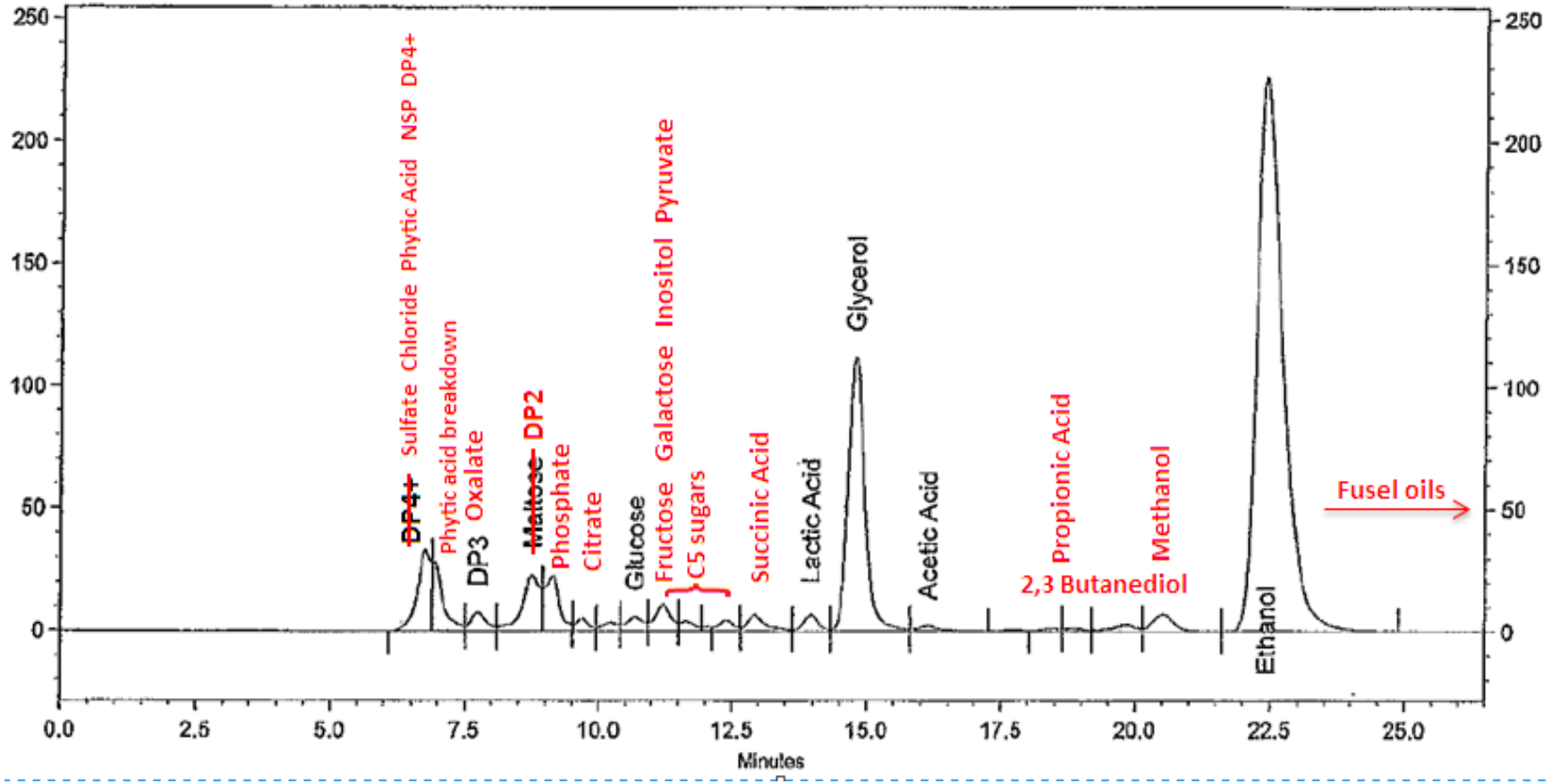
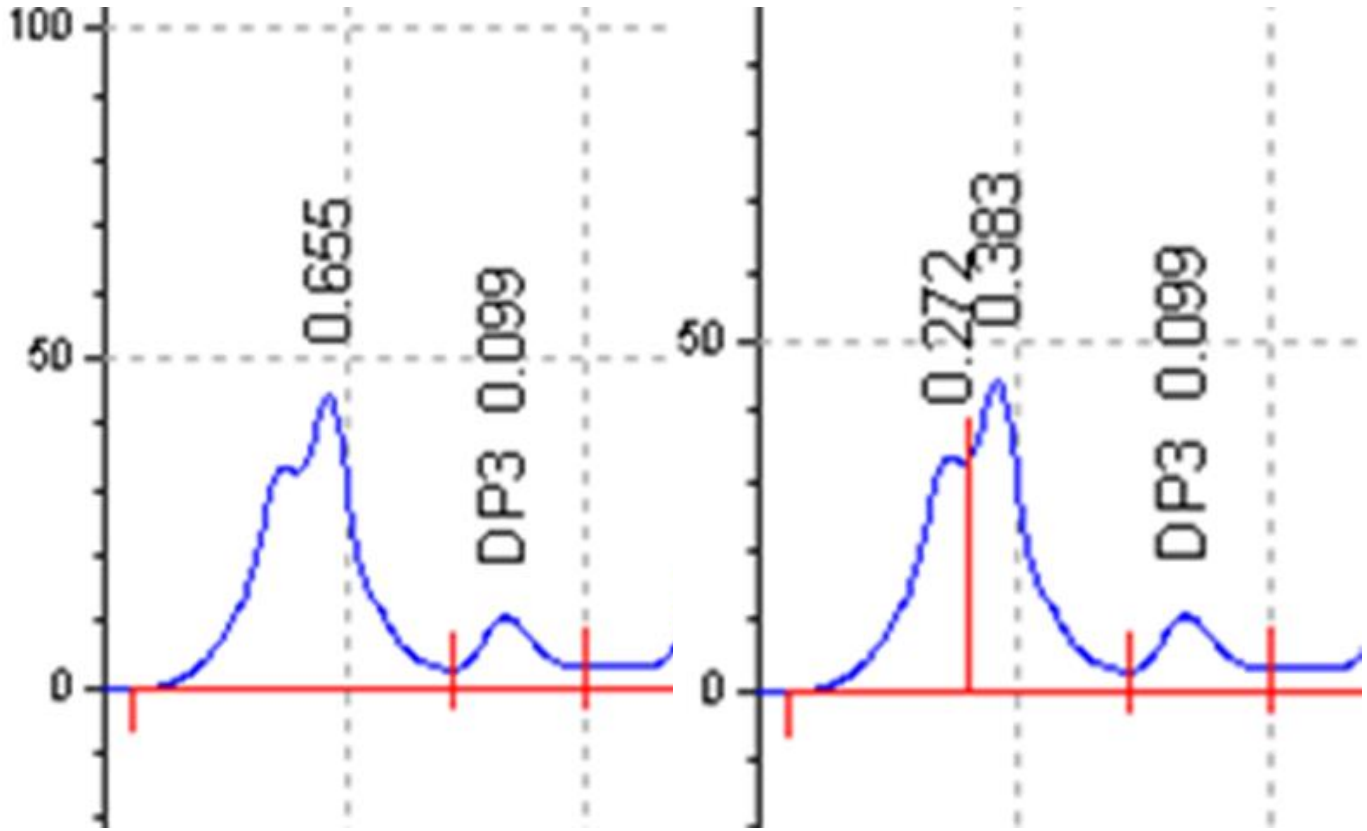


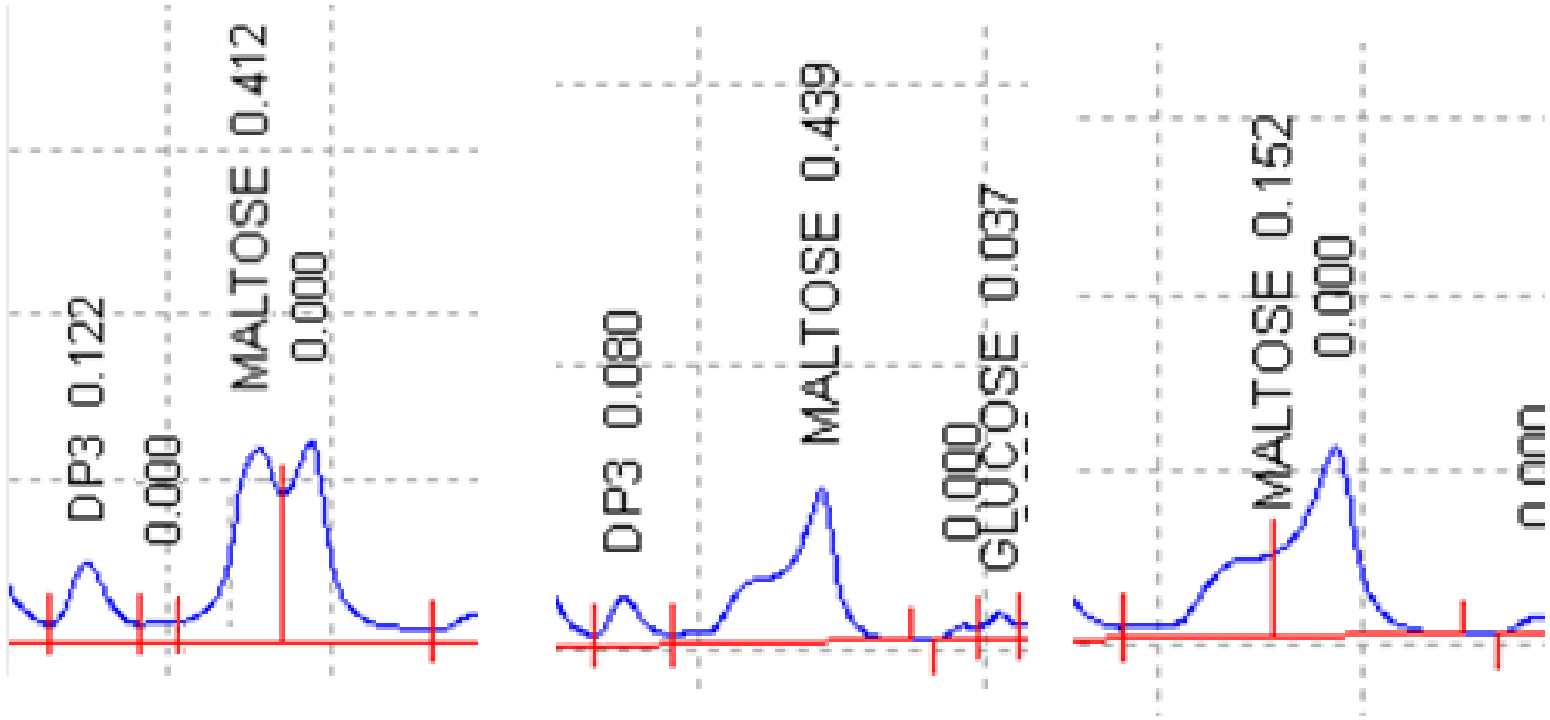
DP4+ INTEGRATION

- **DP4+ peak is sometimes split**
- **Peak splitting can be variable**
- **Peak splitting can change**
  - Especially with phytase treatment
  - Column changes
- **Best practice is to be consistent**
- **Peak groups are helpful in integrating together**



DP2 INTEGRATION

- DP2 and phosphate coelute
- Changes in DP2 or phosphate can change the way the peaks are integrated
- Peak IDs can shift with changing peak sizes



DP2 INTEGRATION OPTIONS

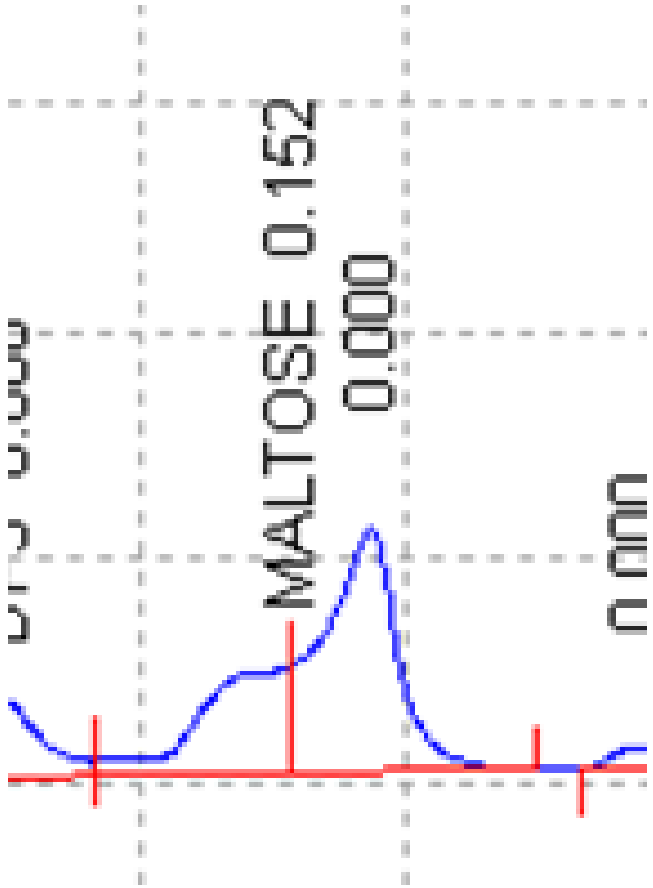
For proper splitting

- Shoulder sensitivity
- Overall sensitivity (threshold/smoothing)
- Apply over set time window if possible

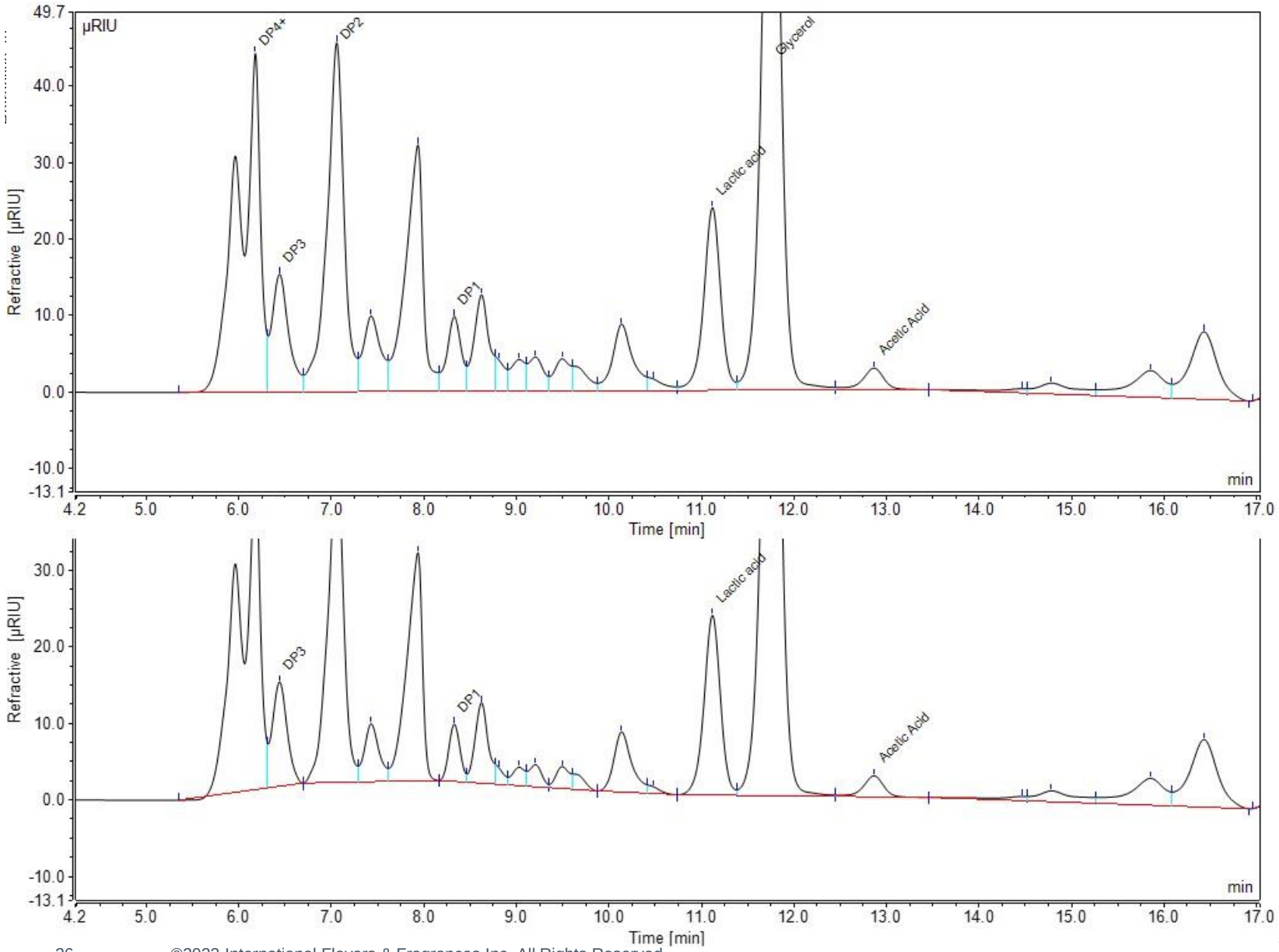
For proper peak IDs

- Change peak windows
- Add phosphate peak

Changes may adversely affect peaks at other time points

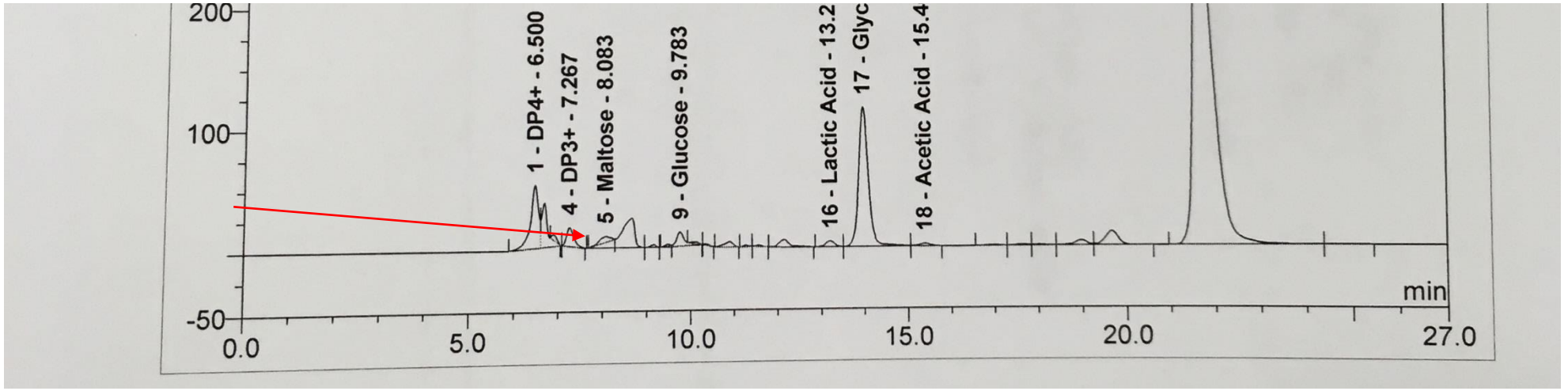


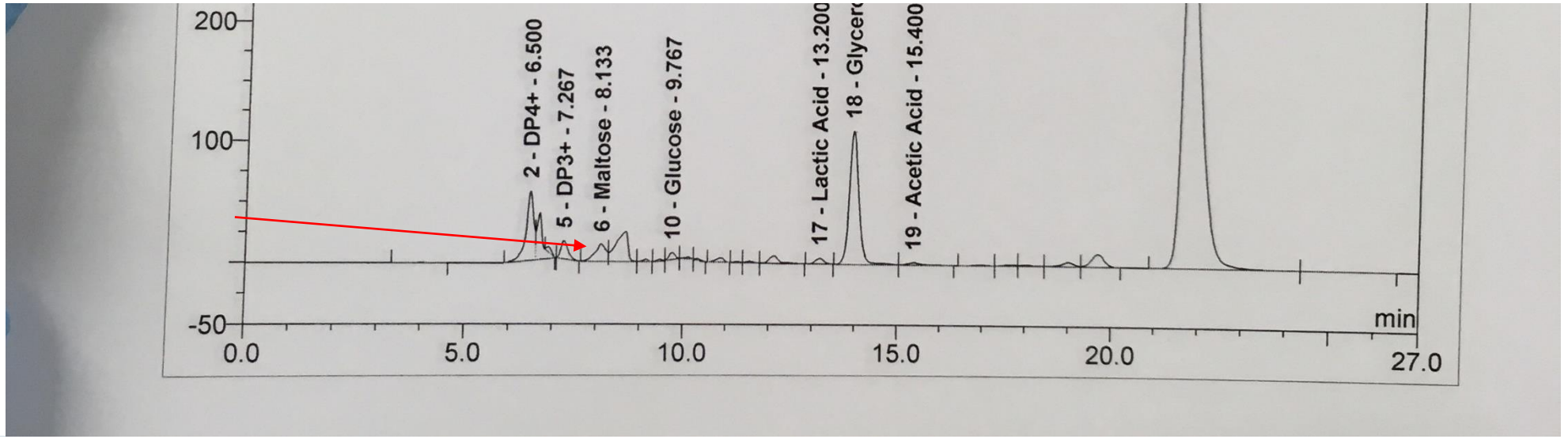
OTHER INTEGRATION DIFFICULTIES- BASELINE CHANGES



Baseline type:	Valley-Valley	Flat
Batch:	4672	4673
DP4+	0.42	0.49
DP3	0.06	0.01
DP2	0.26	0.36
DP1	0.06	0.14
Total sugars	0.8	1
Lactic	0.06	0.07
Glycerol	0.75	0.72
Acetic Acid	0.08	0.13
Ethanol	14.63	14.51

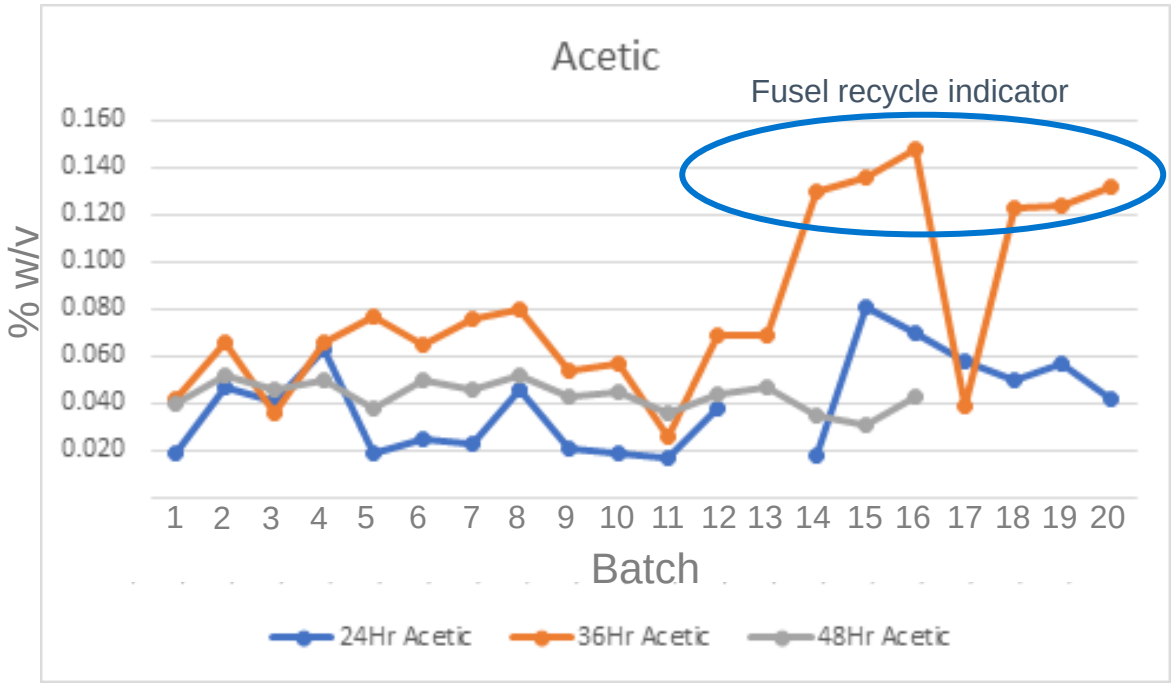
OTHER INTEGRATION DIFFICULTIES- PEAK SHOULDERS





FUSEL INTERFERENCE IN ACETATE PEAK

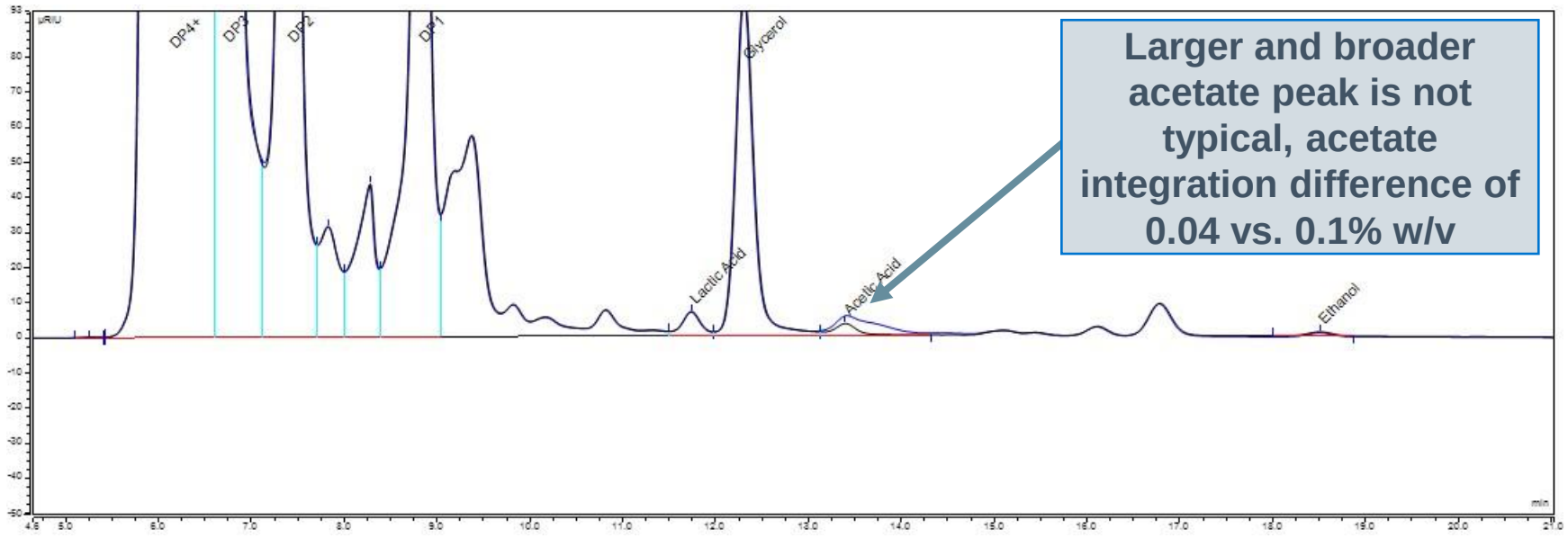
Plant was experiencing sluggish fermentations and seeing unusually high acetate in ferm samples. AIC fusel analysis confirmed fusel recycle issue.



- **Fusels from previous injections carried over into next run**
- **Can elute near acetate and interfere with acetate quantitation**

FUSEL INTERFERENCE IN ACETATE PEAK

- Black: Liquefact injected first run of the day
- Blue: Liquefact injected after drop sample with high fusels



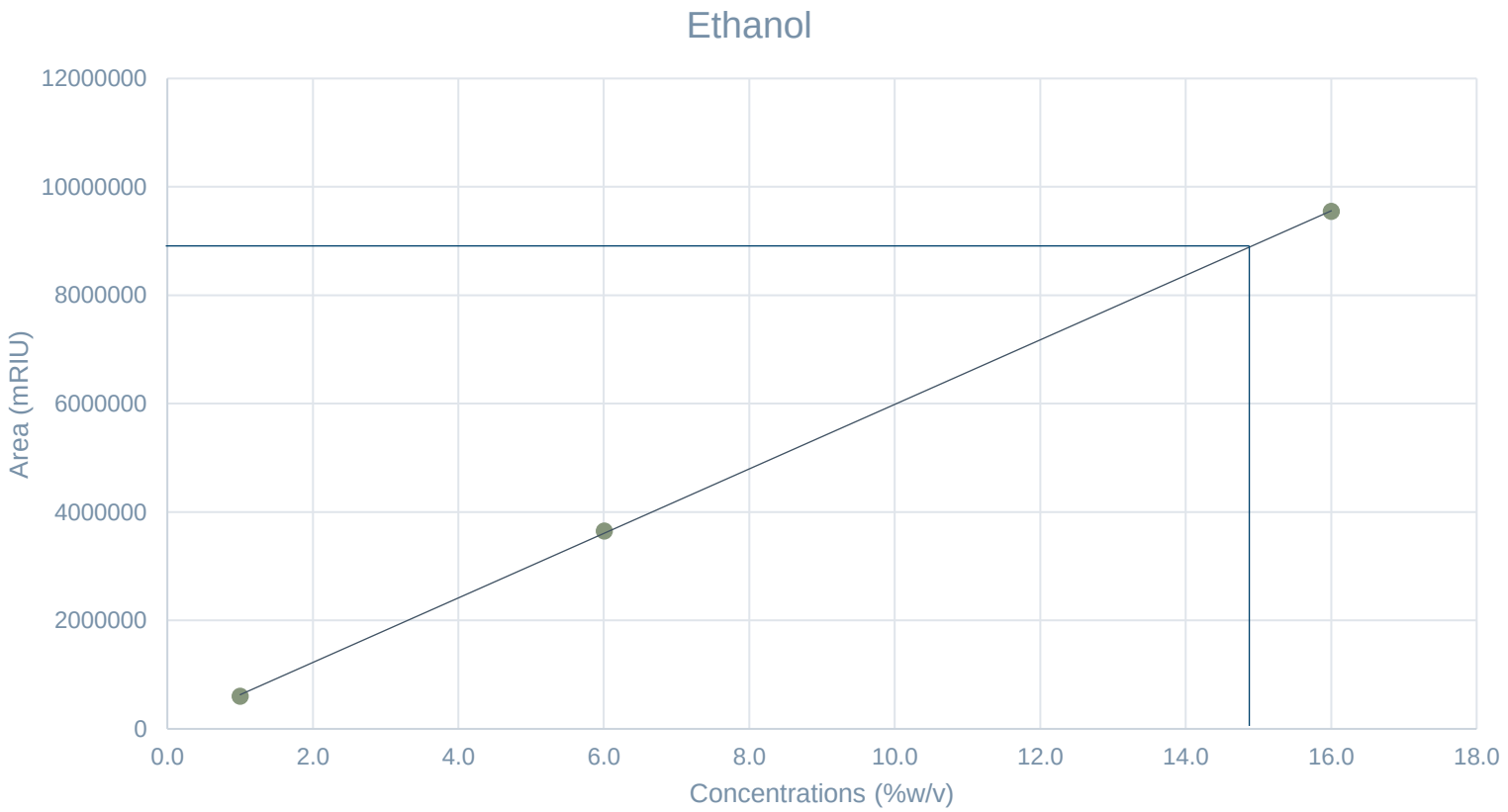
BAD CALIBRATION CURVE

- **Check Standard**
- **Compare areas**
  - Changes in areas should roughly match changes in amounts
- **If areas are similar but amounts are different, there may be a calibration curve issue**
- **In this case, DP4+ was not integrated properly in the Standard**

	Batch	Area	Amount	Area %Δ	Amount %Δ
DP4+	5000	842242	0.433	0	0
	5050	1195681	0.607	42	40
	5075	1064758	0.797	26	84
	5100	1224226	0.933	45	115
DP1	5000	112252	0.084	0	0
	5050	53686	0.039	-52	-54
	5075	50499	0.038	-55	-55
	5100	143093	0.114	27	36
Glycerol	5000	1746929	1.569	0	0
	5050	1685821	1.515	-3	-3
	5075	1811692	1.629	4	4
	5100	1809201	1.625	4	4

BAD CALIBRATION CURVE- ETHANOL

- **Unexpected or drifting results**
- **Sudden change in EtOH values**
  - Especially after a standard change
- **Old standards lose ethanol, but software's calibration level stays the same**
- **5% decrease in high standard area results in sample [EtOH] changing from 15.06% to 15.78%**



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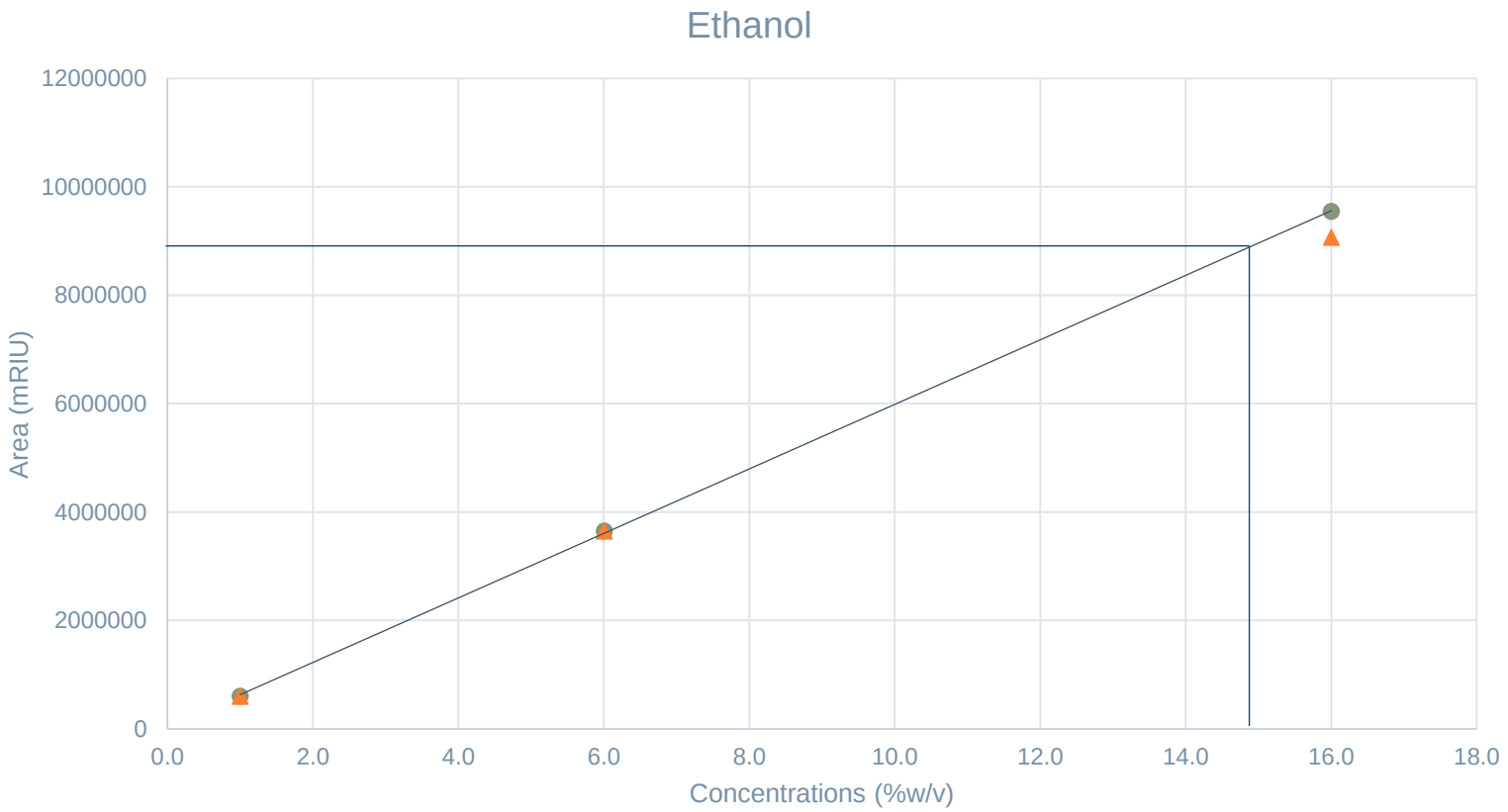

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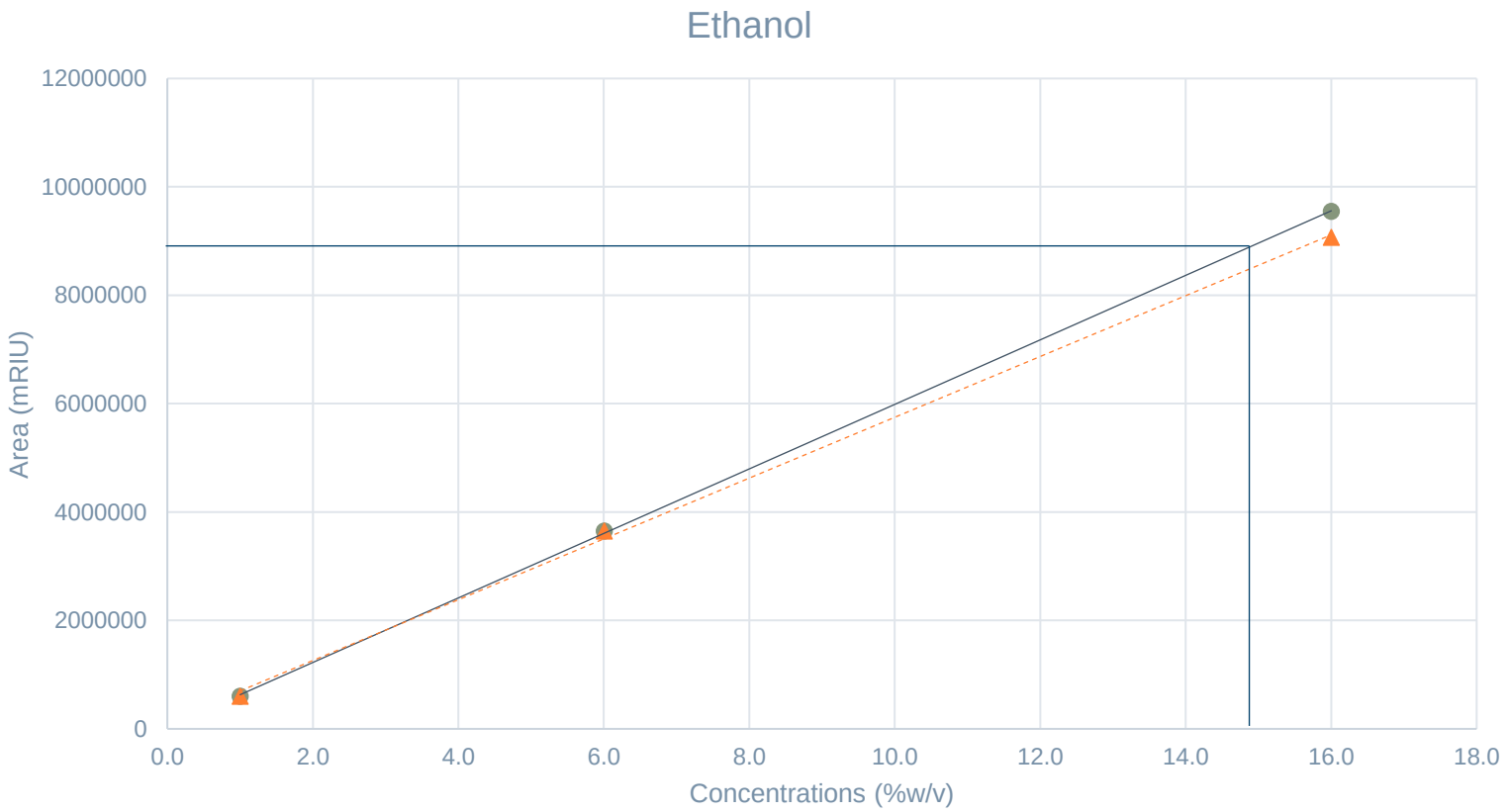

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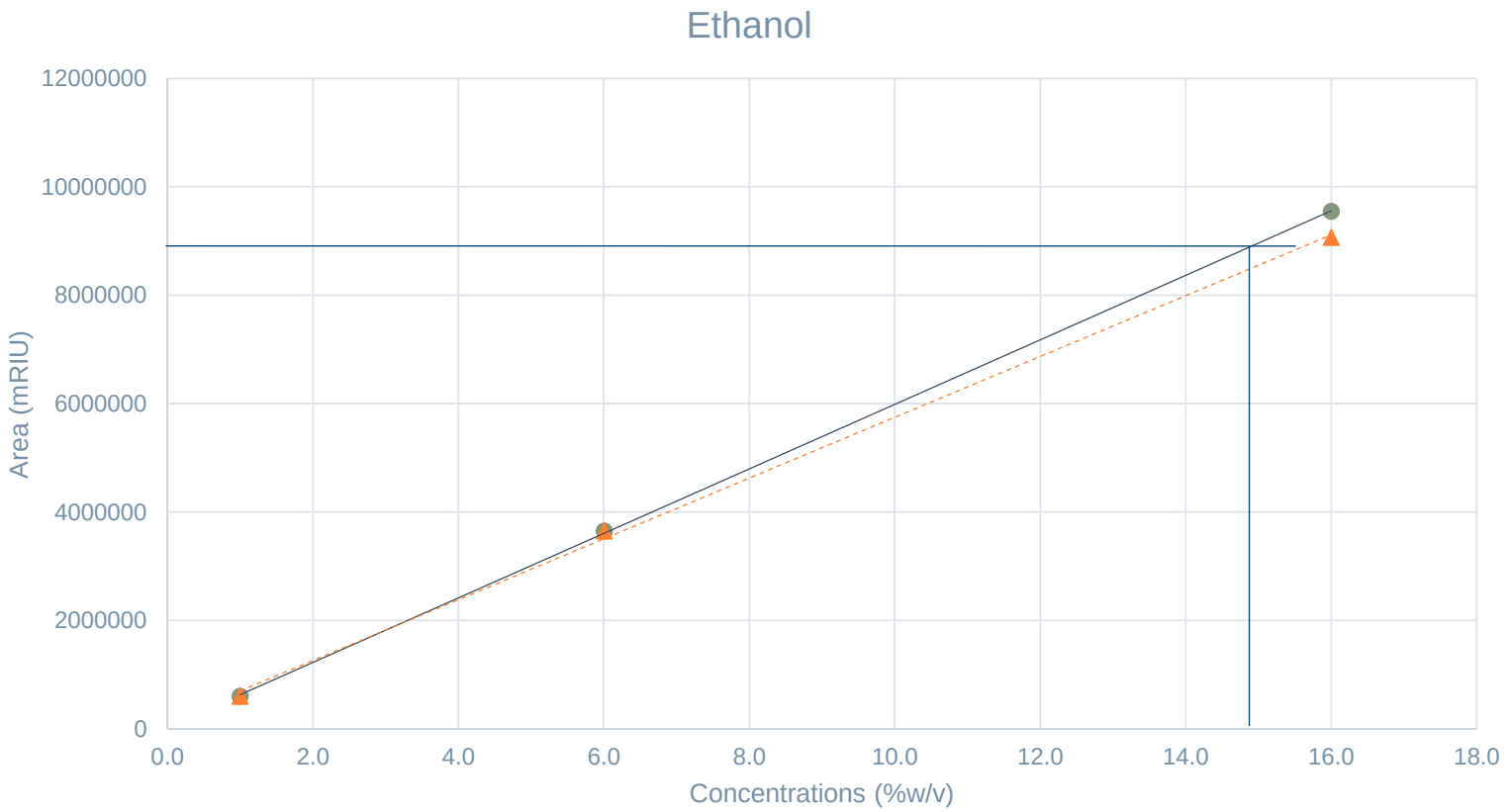

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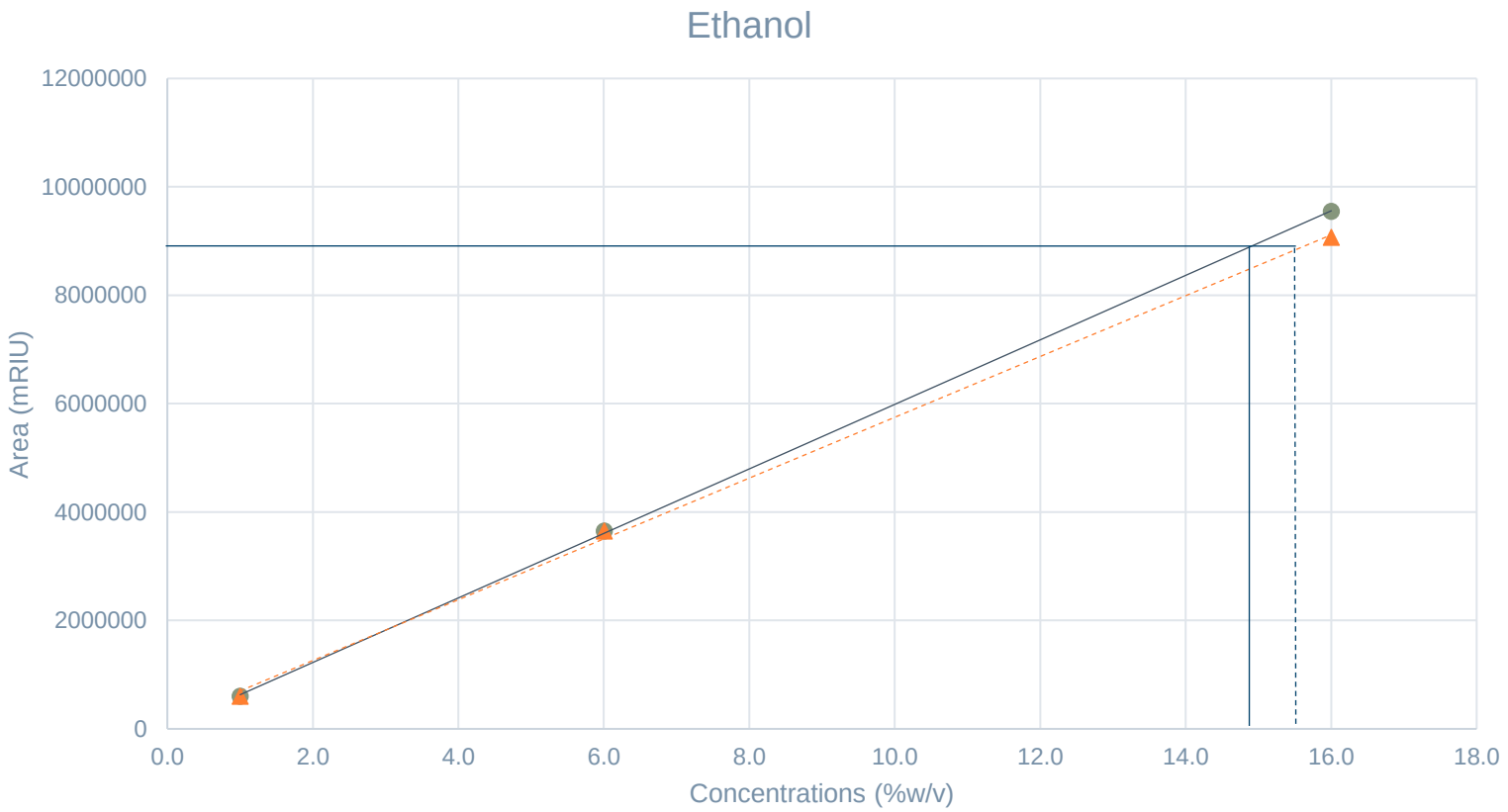
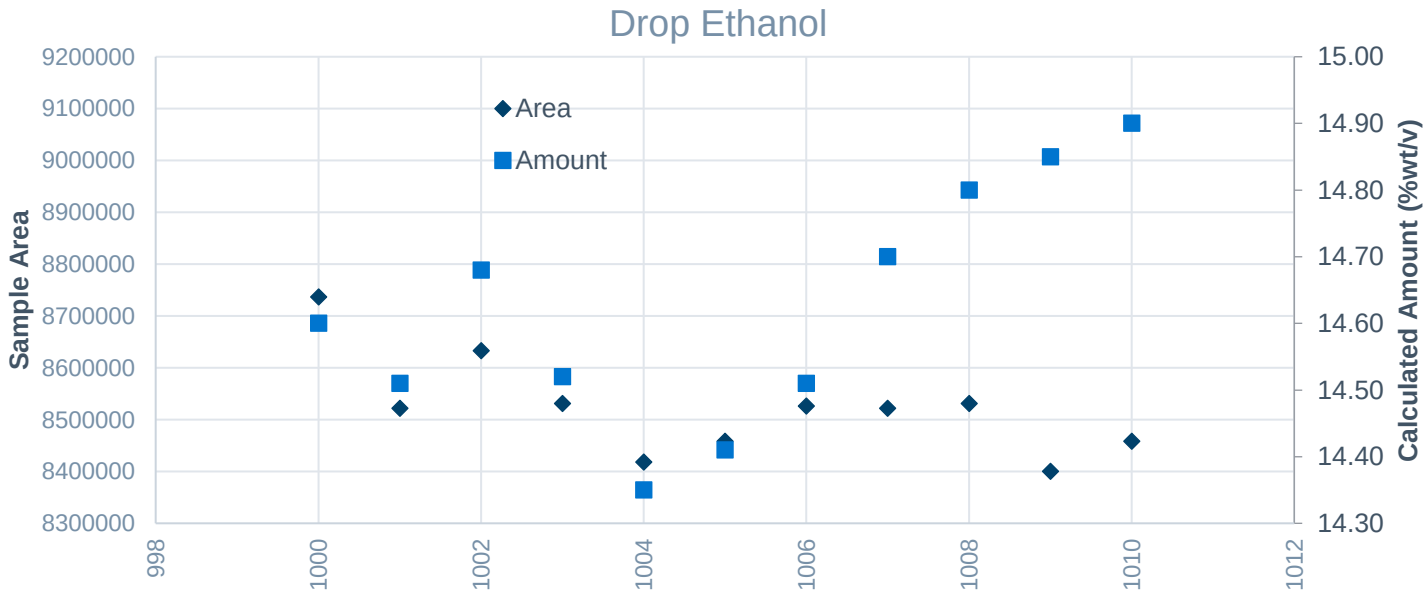


BAD CALIBRATION CURVE- ETHANOL

Resulting Data

Changes in amounts do not correlate with changes in peak area



HPLC PROBLEM CHECKLIST

Identify what is different

Before an issue arises: know what is normal

- Run frequent check standards

After the issue

- Check for physical problems
- Check for integration/peak ID issues
- Check for calibration issues
- Check for reproducibility

If still having issues- note timelines and what could have changed

THANK YOU!

Questions?

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