Analytical method range – what is it and how to maximize it?

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HELLO!

I am Dr. Ilkka Lahdesmaki

Chief Scientist

You can find me at ilkka@flowinjection.com

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DEFINITION OF RANGE

DEFINITION

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IUPAC Gold Book (goldbook.iupac.org)

- Dynamic range = “The ratio between the maximum usable indication and the minimum usable indication (**detection limit**). A distinction may be made between the **linear dynamic range**, where the response is directly proportional to concentration, and the **dynamic range** where the response may be non-linear, especially at higher concentrations.”

DEFINITION

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Eurachem Validation Guide (www.eurachem.org)

- "The 'working range' is the interval over which the method provides results with an **acceptable uncertainty**. The lower end of the working range is bounded by the limit of quantification **LOQ**. The upper end of the working range is defined by concentrations at which **significant anomalies** in the analytical sensitivity are observed."
- Differentiates between **instrument working range** and **method working range**

DEFINITION

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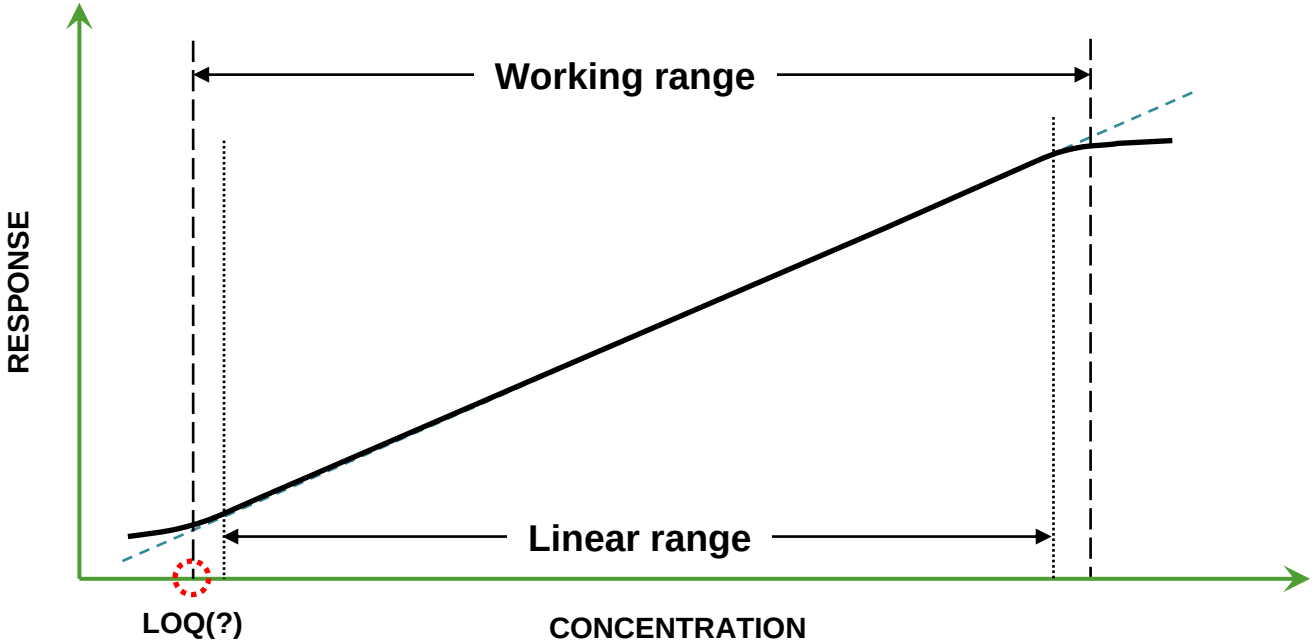
- ICH guidelines, 1994-2005 (database.ich.org)
  - Range "… is established by confirming that the analytical procedure provides an acceptable degree of **linearity, accuracy and precision** when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure."
- ICH guidelines, 2022 draft (www.ema.europa.eu)
  - Mentions different cases: linear response, non-linear response, multivariate calibration
  - Defining a range: general considerations, little specifics

DEFINITION



DEFINITION

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- Instrument working range vs. method working range
  - TKN: detect ammonia, but digestion and impurities affect range
  - TN: detect nitrate, impurities in persulfate affect range

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WHAT DETERMINES RANGE

Low end & high end

WHAT DETERMINES RANGE

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Low end (“What determines LOD/LOQ”)

- Signal strength (e.g., color intensity)
- Instrument parameters (e.g., sample volume)
- Chemical noise (e.g., baseline wobble)
- Electronic noise – detector (light source, flow cell, sensor)
- Electronic noise – A/D converter
- Blank variability

SIGNAL

NOISE

WHAT DETERMINES RANGE

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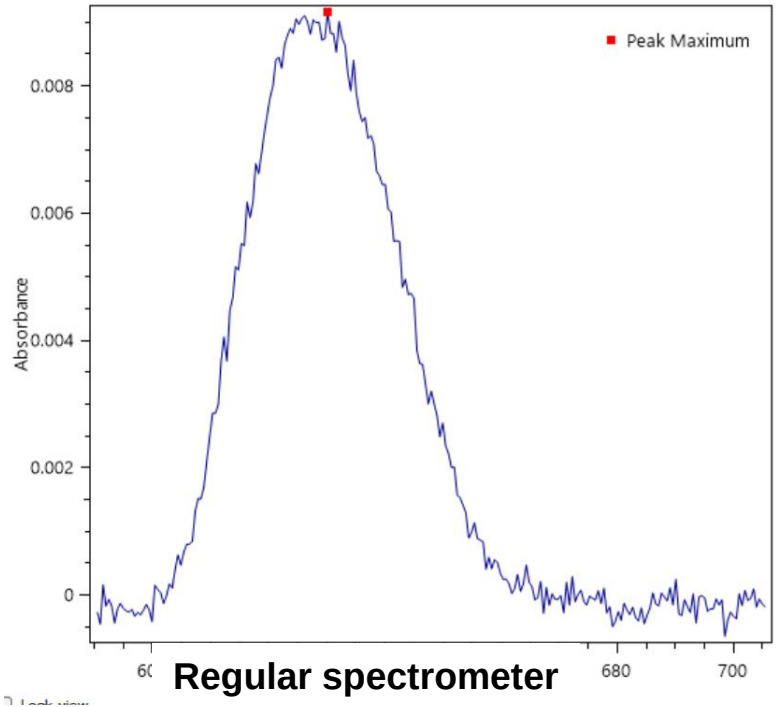
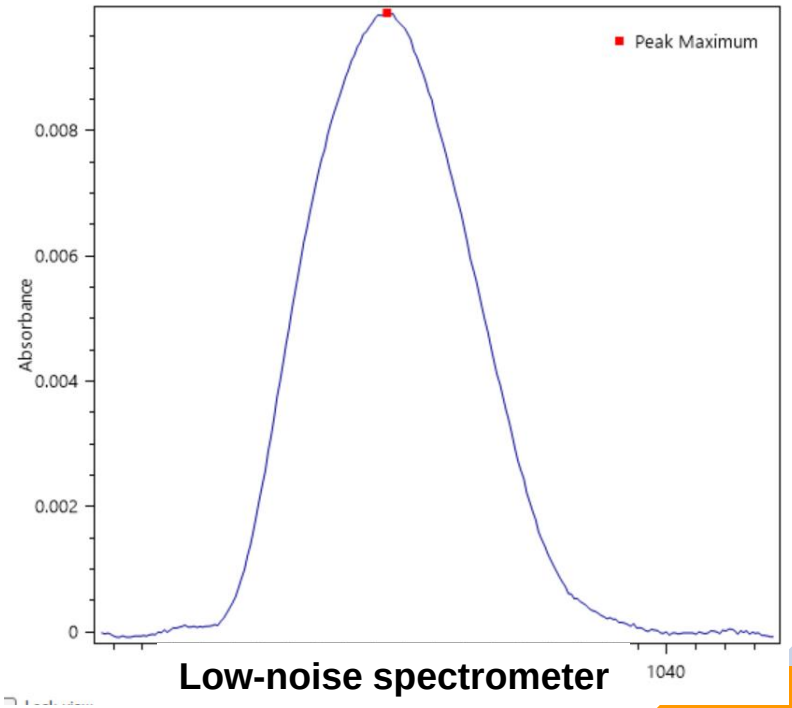


Regular spectrometer



Low-noise spectrometer

GD NH3
0.1 mg N/L

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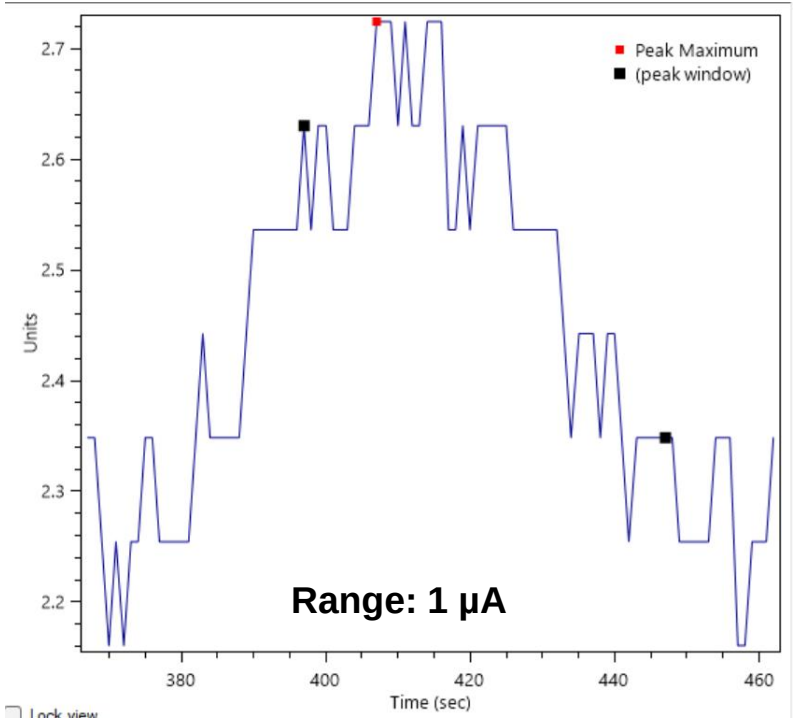

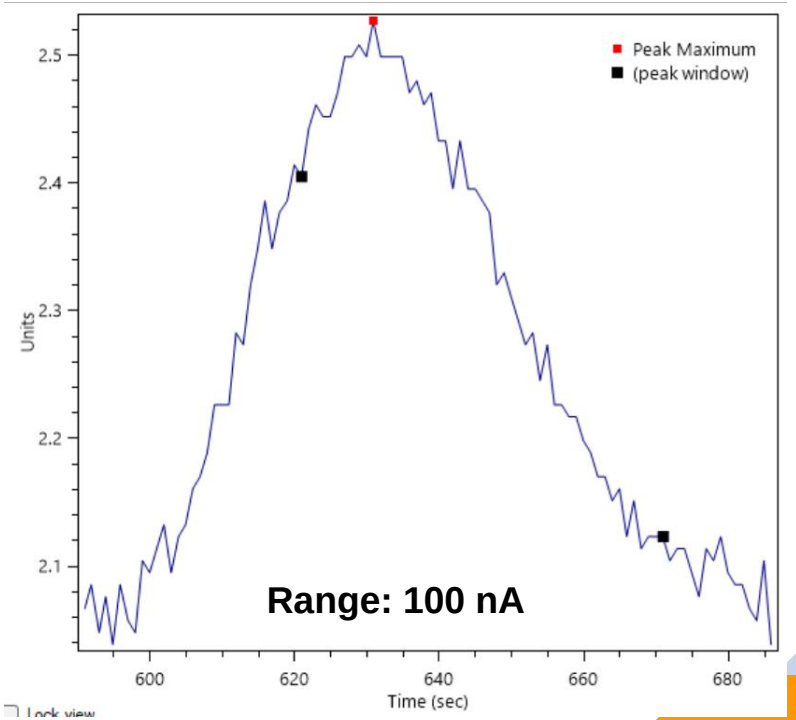

AMP CN
1 µg/L

WHAT DETERMINES RANGE

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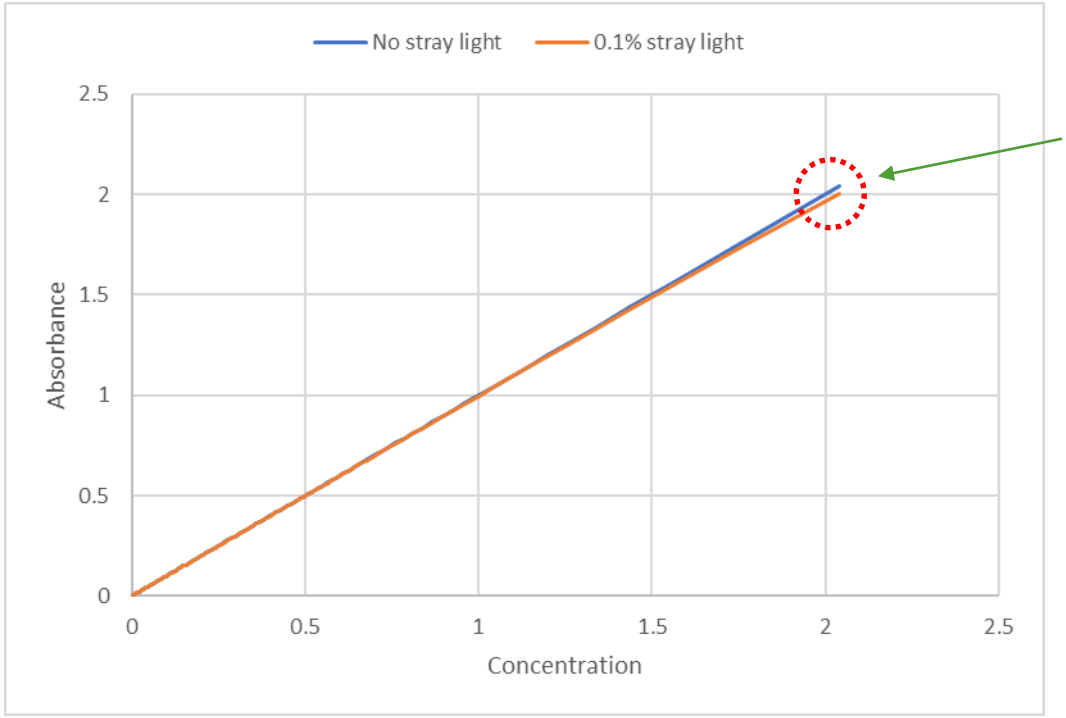
- High end
  - Chemical depletion (limiting reagent), chemical reaction
  - Instrument parameters (e.g., sample volume, detector optical path)
  - Detector limitation (e.g., stray light)
  - Electronic limitation (e.g., A/D range)

WHAT DETERMINES RANGE



DEVIATION AT HIGH A.U. IN PRESENCE OF STRAY LIGHT

WHAT DETERMINES RANGE

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Back-of-the-envelope calculation: absorbance detector

- Noise on a great absorbance detector ~10 µAU
- For LOQ, need to be at ~10× noise ⟶ ~100 µAU = 0.1 mAU = 0.0001 AU
- Upper range of a good absorbance detector ~2 AU
- Widest possible range ~ 2 AU / 0.0001 AU = 20,000 ~ theor. max 4 orders of magnitude
- Realistic max range probably a little over 3 orders of magnitude

WHAT DETERMINES RANGE

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- Back-of-the-envelope calculation: fluorescence by PMT
  - Use a PMT in “photon counting mode”
  - Measure increase of light, not decrease of light → no hard ceiling like in absorbance
  - Digitization based on a counter instead of A/D → no “bit resolution” issue
  - Can handle ~5 orders of magnitude

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HOW CAN RANGE BE EXTENDED?

Specifically for the high end

HOW CAN RANGE BE EXTENDED

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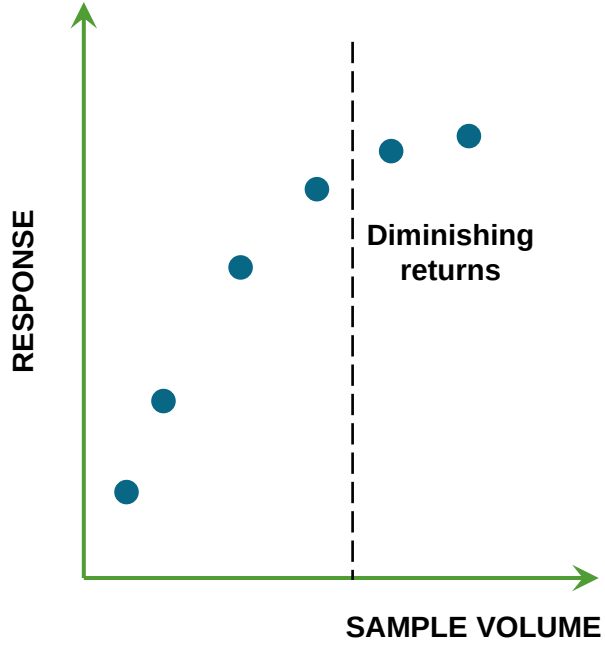
- "Lazy answer": automated dilution
  - Easy – but also **slow**
- Adjust sample loop size (FIA, LC, IC)
- Adjust optical path on flow cell
- Use alternate wavelength (absorbance detection)
- Use a different detection principle
- In-line dilution (dilute while previous sample measured)

HOW CAN RANGE BE EXTENDED

- Adjust sample loop size
  - A very simple & efficient way to adjust range
  - Scales proportionately for low volumes, diminishing returns for high volumes
  - Avoid extremes
    (in FIA, 30 µL - 300 µL is a safe range)



HOW CAN RANGE BE EXTENDED

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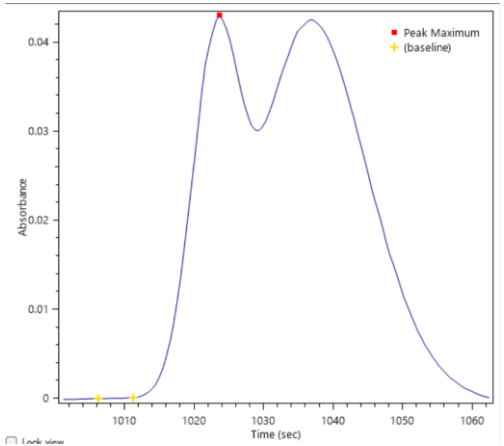
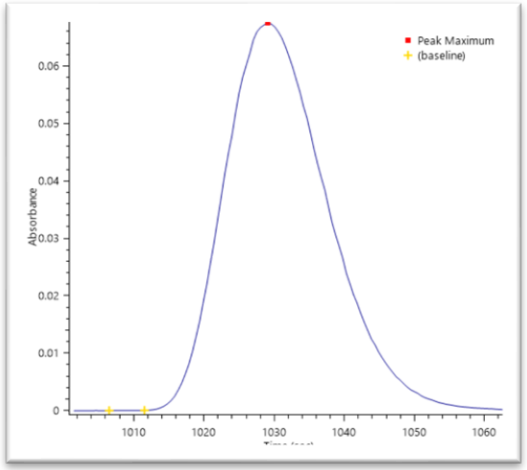


Split peak due to high acidity

Smaller sample loop



HOW CAN RANGE BE EXTENDED

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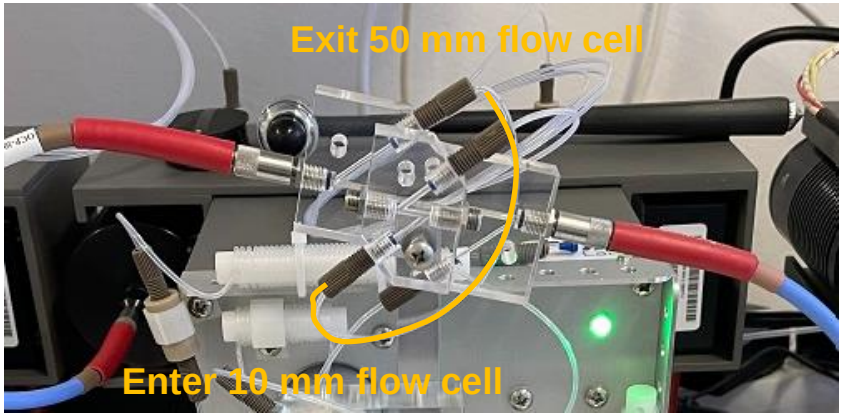
- Adjust optical path on flow cell
  - Another simple & efficient route
  - Again, avoid extremes
    (in FIA, 2.5-50 mm is a good range)



HOW CAN RANGE BE EXTENDED

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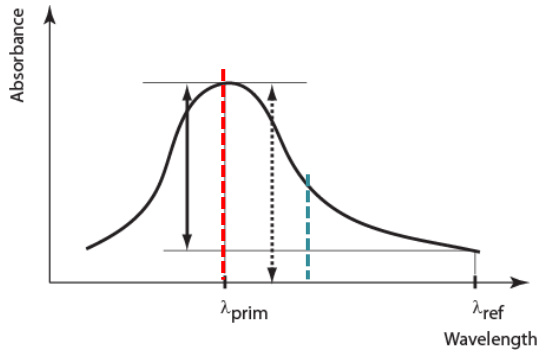
Alternate detection wavelength

- Wavelength off of absorbance max ⟶ lower response
- Only possible with array-type detectors (CCD, PDA)



HOW CAN RANGE BE EXTENDED

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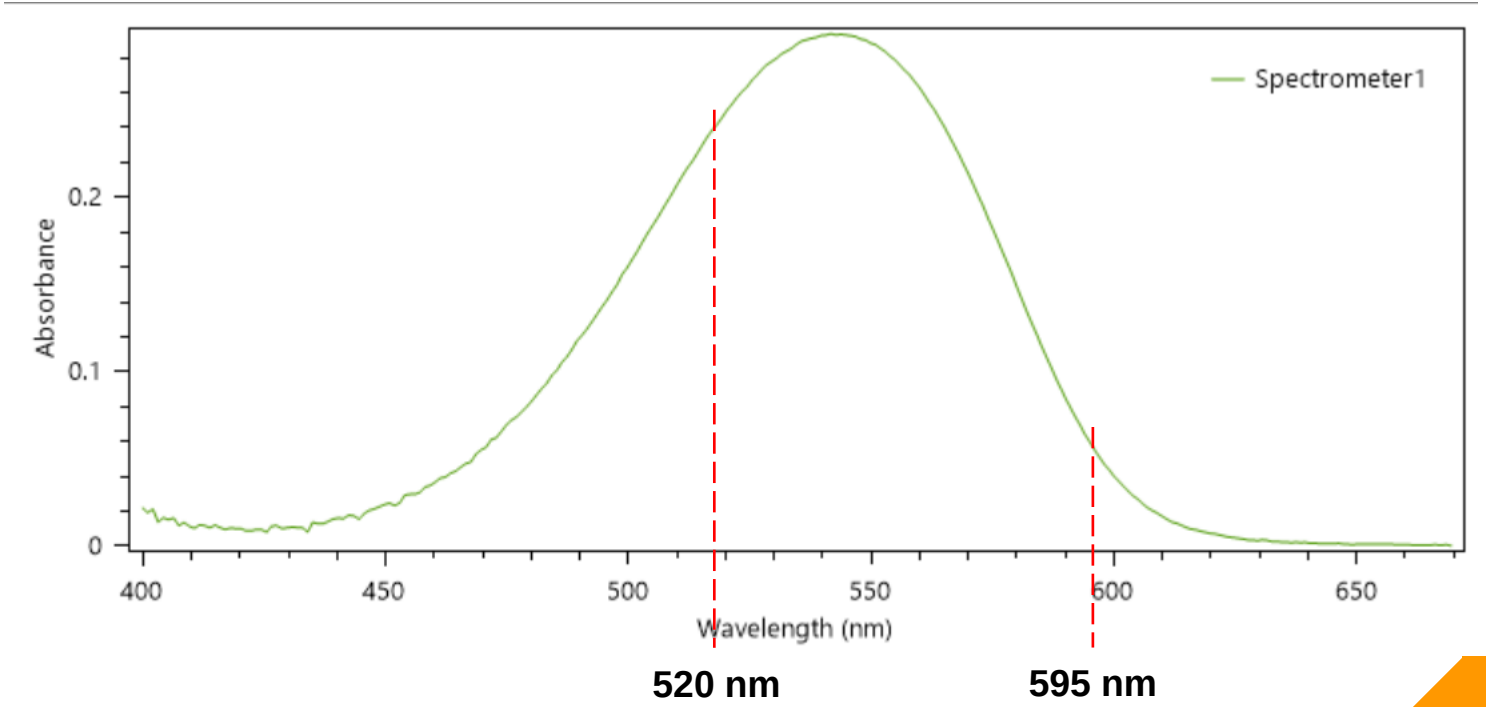

Colorimetric NOx method

HOW CAN RANGE BE EXTENDED

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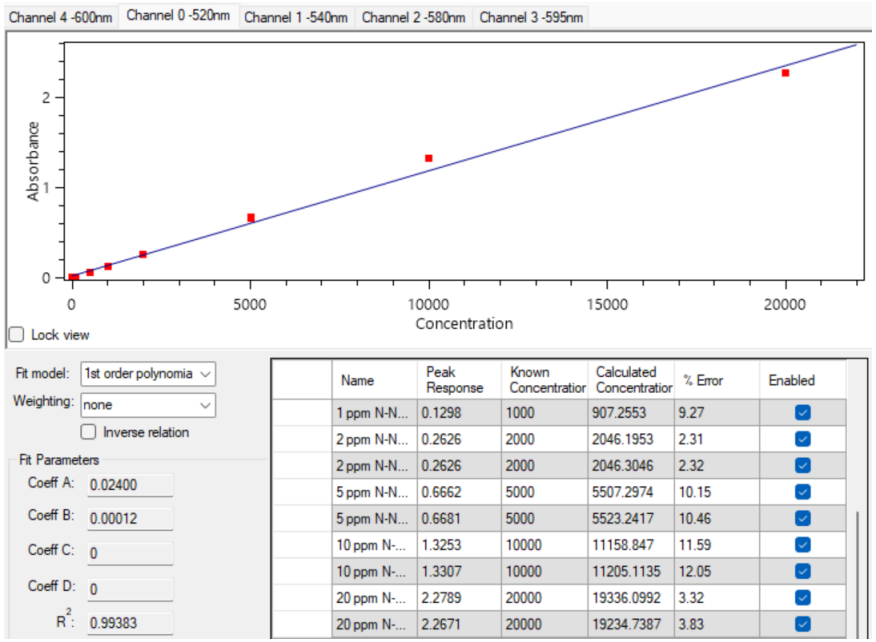
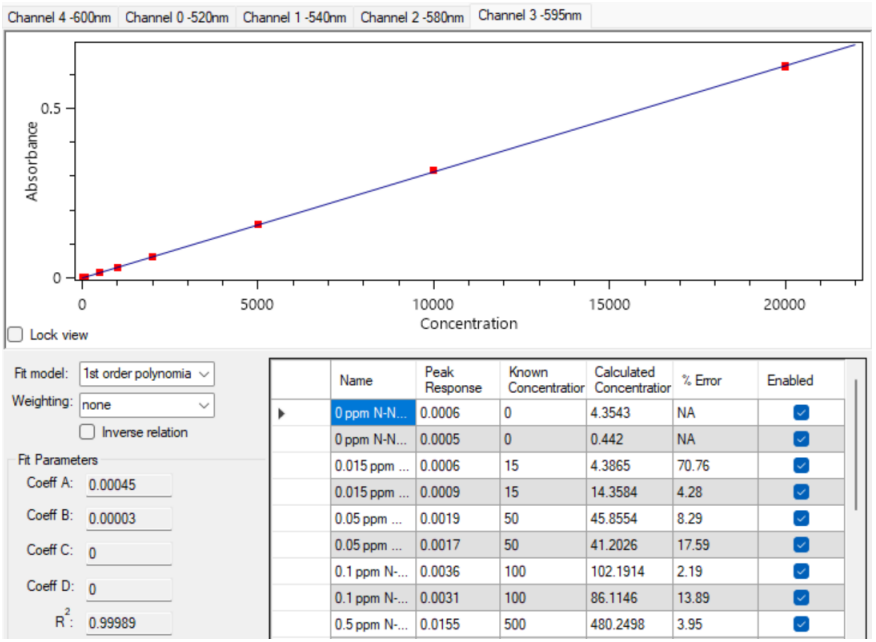


520 nm



595 nm

HOW CAN RANGE BE EXTENDED

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- 40 CFR §136.6 allows the use of alternate wavelengths

> (xx) Changes in equipment operating parameters such as the monitoring wavelength of a colorimeter or the reaction time and temperature as needed to achieve the chemical reactions defined in the unmodified CWA method. For example, molybdenum blue phosphate methods have two absorbance maxima, one at about 660 nm and another at about 880 nm. The former is about 2.5 times less sensitive than the latter. Wavelength choice provides a cost-effective, dilution-free means to increase sensitivity of molybdenum blue phosphate methods.

HOW CAN RANGE BE EXTENDED

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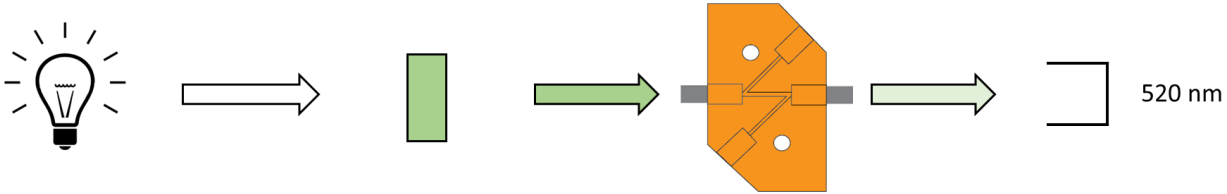
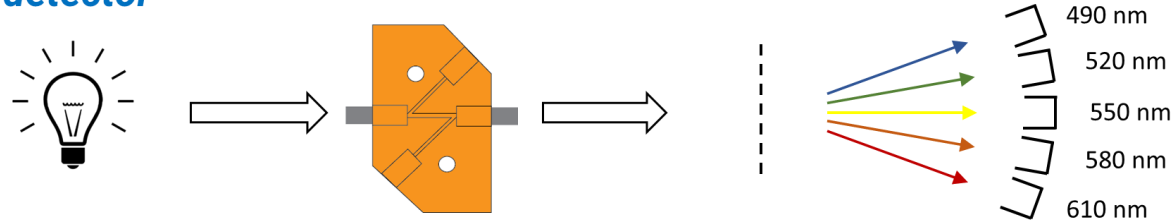
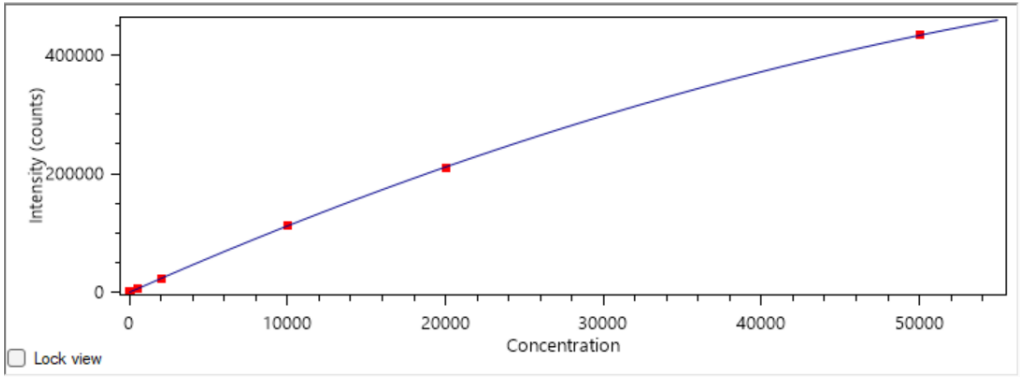
Traditional photometric detector



Array detector



HOW CAN RANGE BE EXTENDED



Fit model: 2nd order polynomi
Weighting: 1/response
Inverse relation

Fit Parameters

Coeff A:	137.60005
Coeff B:	11.82542
Coeff C:	-0.00006
Coeff D:	0
R^2:	0.99997
RSE (%):	2.75292

	Name	Peak Response	Known Concentration	Calculated Concentration	% Error	Enabled
▶	Std1	137.6000	0	0	NA	☑
	Std2	741.1333	50	51.0509	2.10	☑
	Std3	1369.4000	100	104.2234	4.22	☑
	Std4	5948.3333	500	492.672	1.47	☑
	Std5	23048.1333	2000	1957.8541	2.11	☑
	Std6	111498.4000	10000	9944.876	0.55	☑
	Std7	209786.8500	20000	19826.5121	0.87	☑
	Std8	435405.1333	50000	50321.9795	0.64	☑

Fluorescence-based NH3 method

HOW CAN RANGE BE EXTENDED

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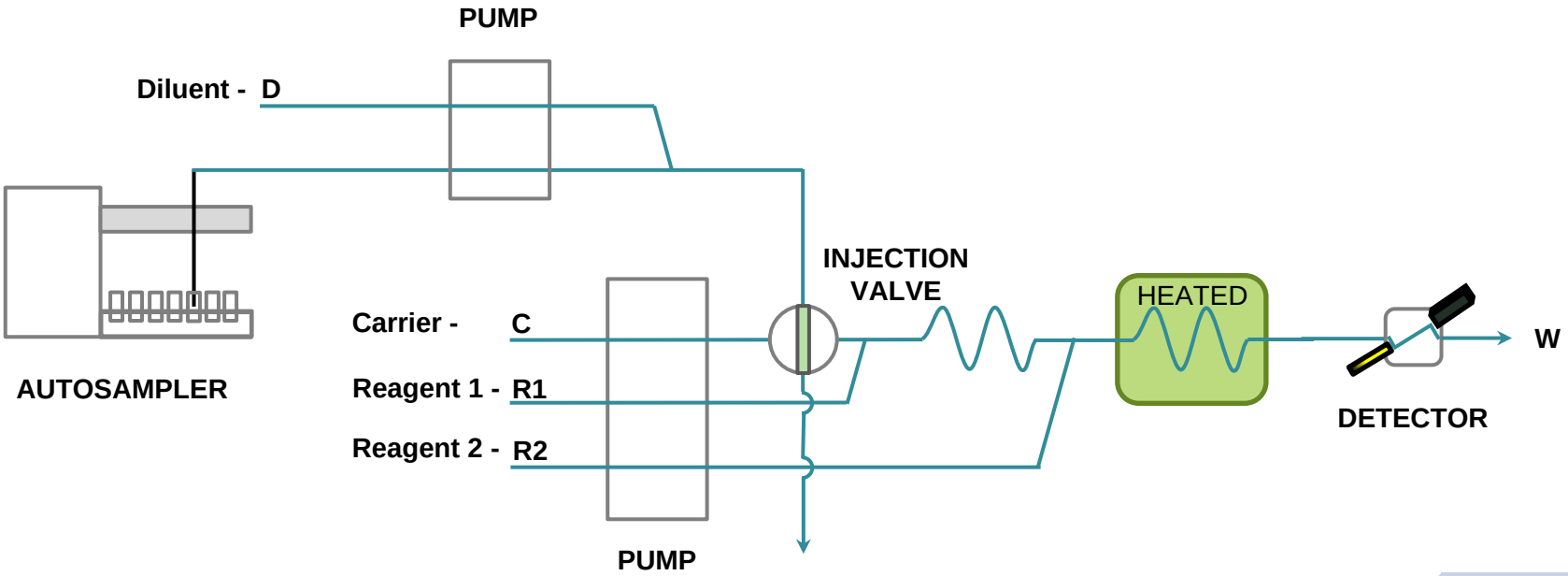

CONCLUSIONS

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- Distinction between instrument range and method range
- “Moderation in everything” is a good guideline
- Understanding how instruments and methods work helps you in range adjustment
- In most cases, 3 orders of magnitude is the practical limit
- Use of alternate detection wavelength is a “penalty-free” way of extending range

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THANKS!

Any questions?

You can find me at
sales@flowinjection.com