Deep UV Raman and fluorescence control for Continuous flow manufacturing

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IFPAC
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Deep UV Raman & Fluorescence Spectroscopy for In Situ Process Analysis

- **Problem**:
  - NIR and FITR techniques are limited in most powder mixtures to > 2% bulk ratios. Absorption techniques and excipient interferents limits dynamic range.
- **Goal**:
  - Explore other techniques focused in Deep UV spectroscopy (fluorescence and Raman) to extend in process control of high potency drugs to better than 0.1% bulk ratios.
- **Solution**:
  - A handheld size, deep UV Raman/Fluorescence instrument, that avoids spectral obscuration enabling the advantage of both spectroscopic techniques.

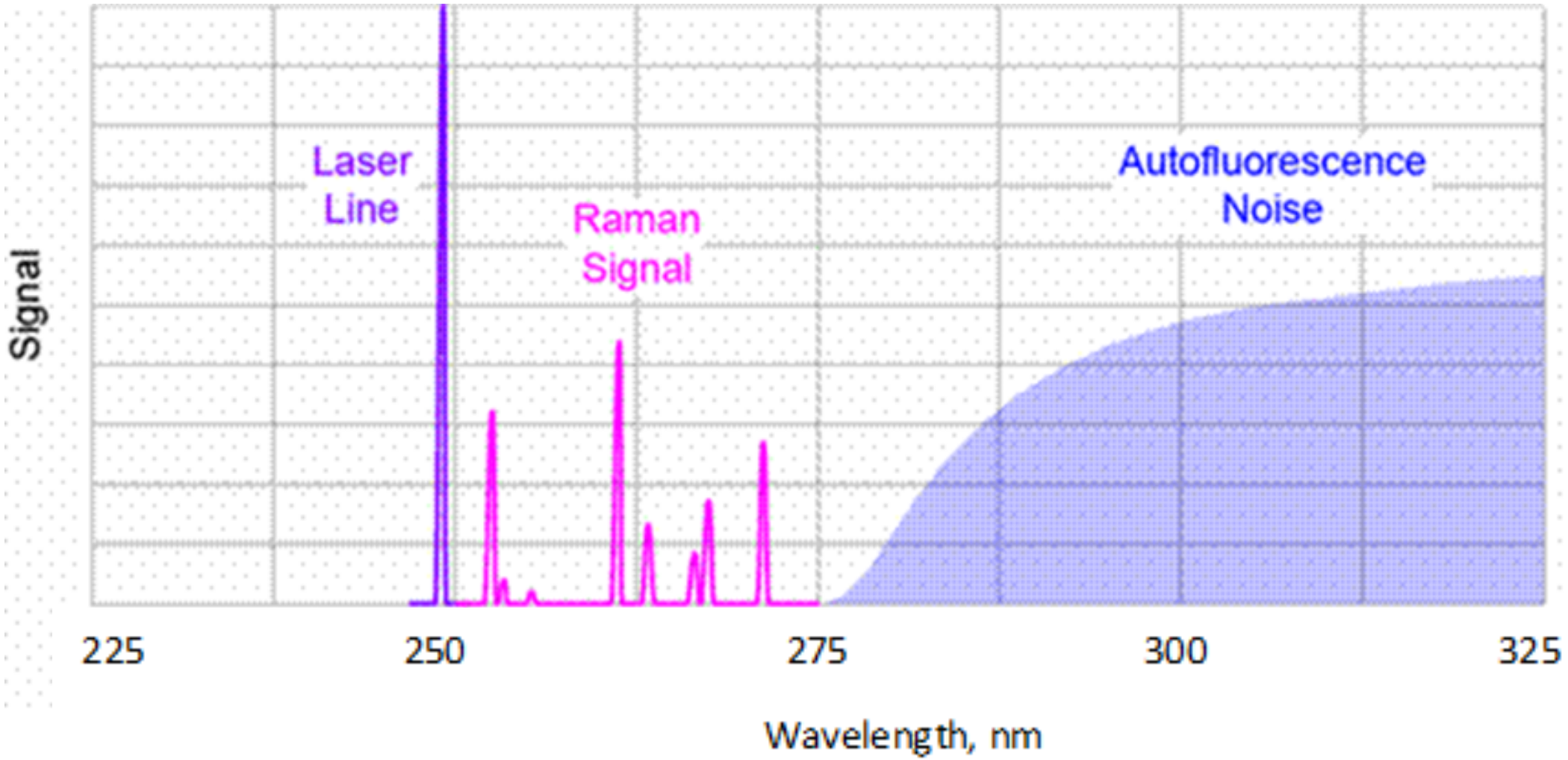
Advantages of deep UV Raman & fluorescence detection

Advantages of Deep UV Detection vs Visible or IR?

- Non-contact, reagentless, no sample handling or preparation
- Excitation below 250 nm separates Raman & fluorescence spectral regions to enable
  - ✓Clear Raman spectra with no obscuration or alteration by native fluorescence
  - ✓No alteration of the fluorescence spectra by major Raman bands
  - ✓The ability to simultaneously detect Raman and native fluorescence
- Much higher Raman sensitivity due to Rayleigh law and resonance Raman enhancement effects
- Fluorescence detection alone has much higher specificity when excitation is below 250 nm
- Detection of concentration of pharma materials in the low ng/cm2 has been demonstrated
- Detection is solar blind, enabling detection in full daylight without interferences

Separation of Rayleigh, Raman, & Fluorescence

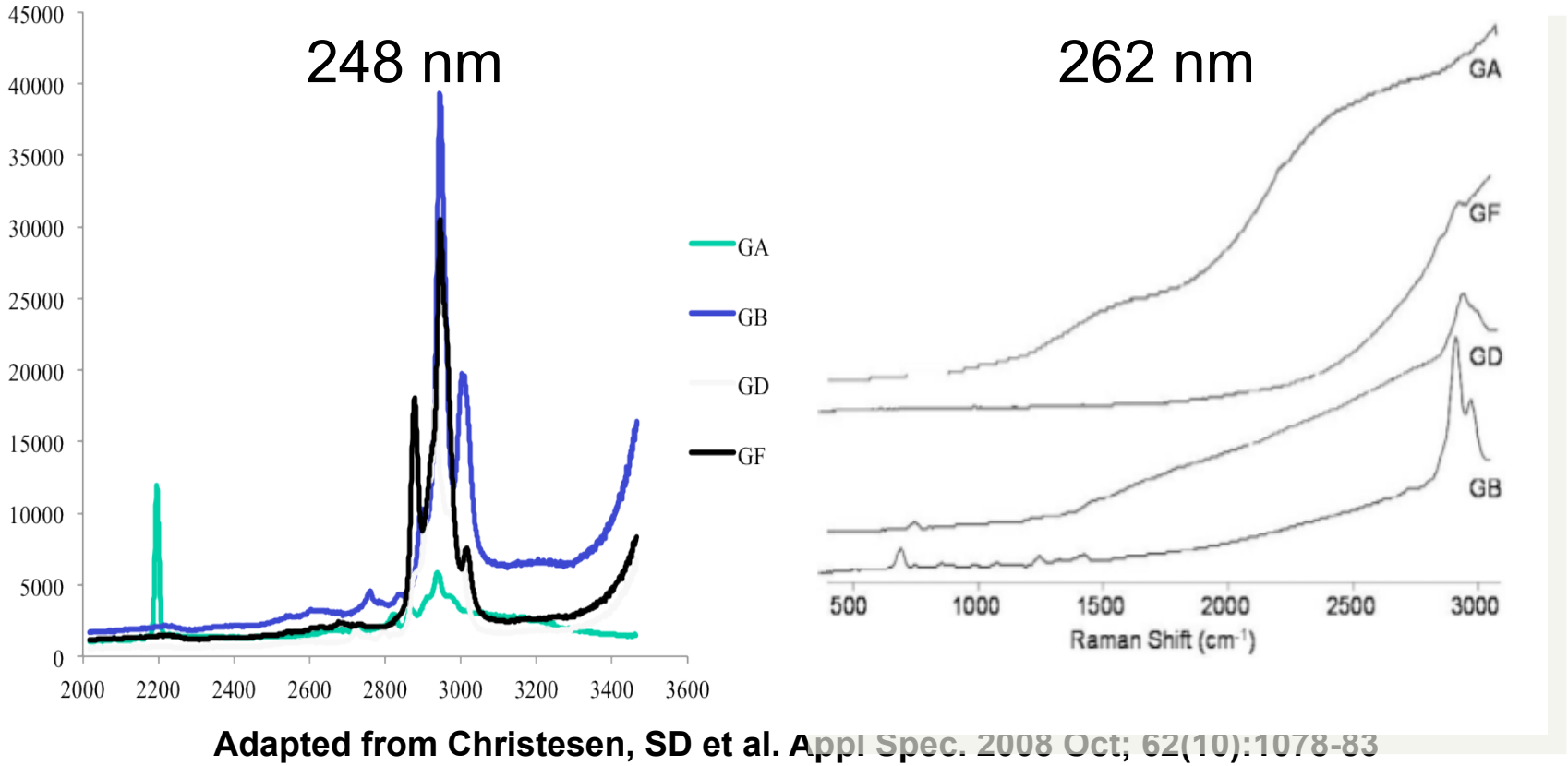
when excitation is < 250 nm



Sensitivity to Excitation Wavelength

Raman Spectra with Excitation at 248 nm versus 262 nm

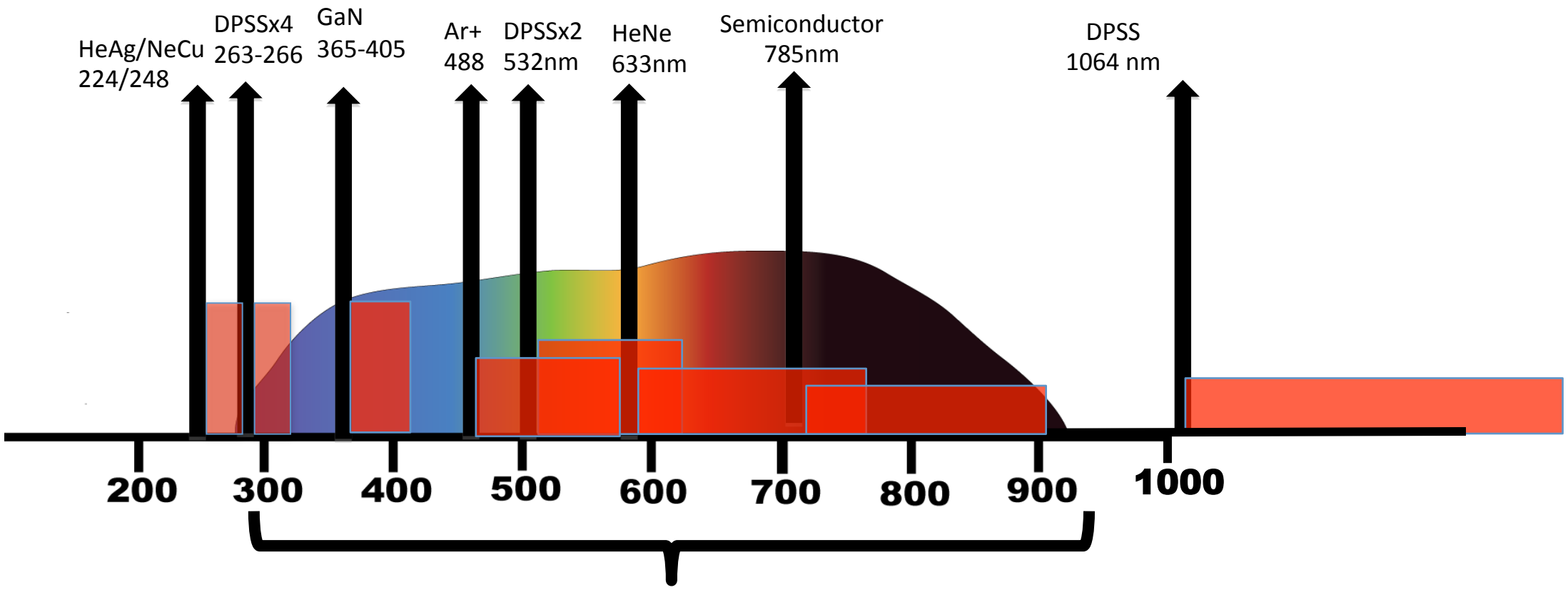
(Example is G Agents)



Adapted from Christesen, SD et al. Appl Spec. 2008 Oct; 62(10):1078-83

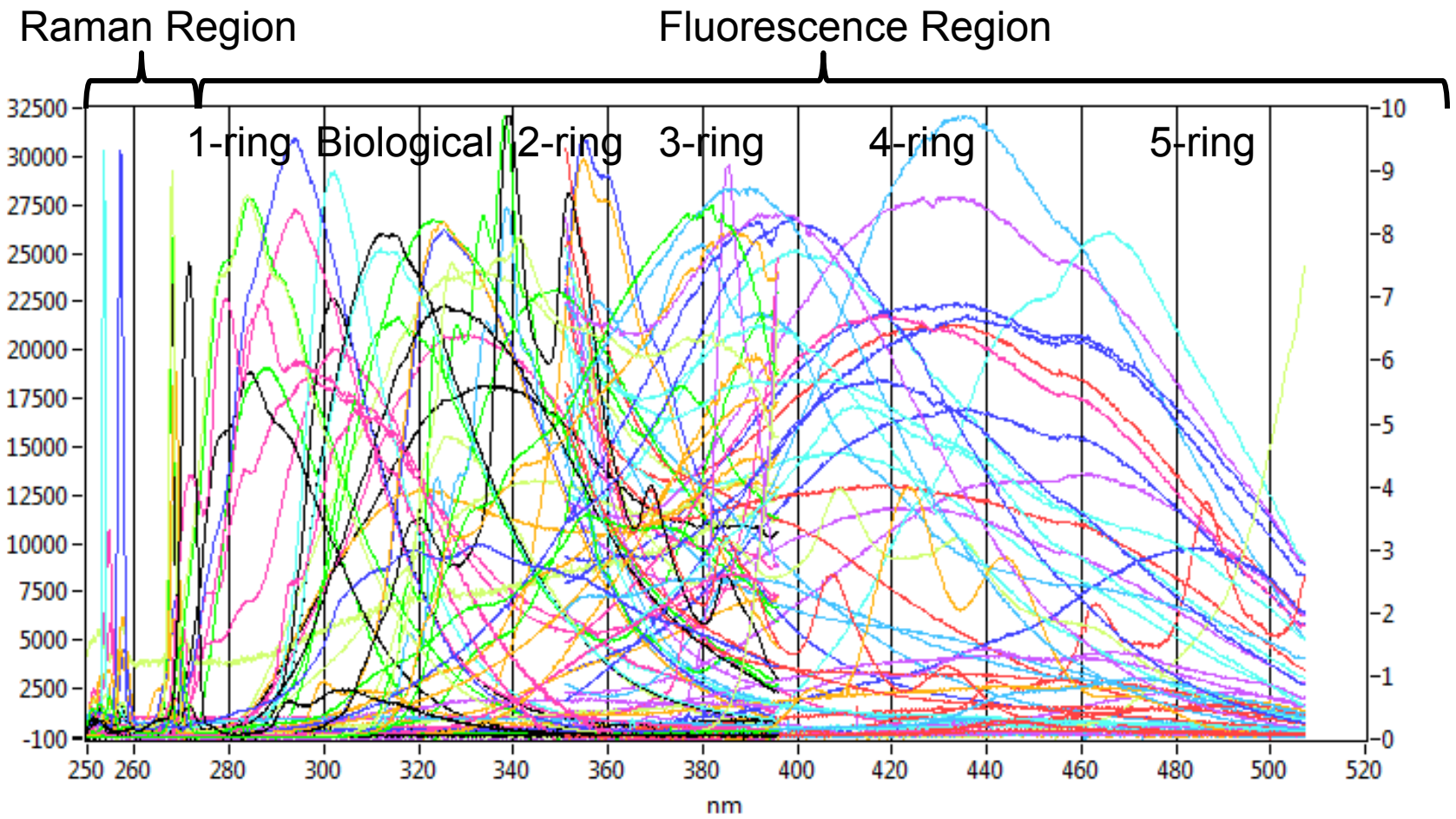
Why Deep UV below 250nm?

When excitation < 250nm Raman and fluorescence spectral regions are separated



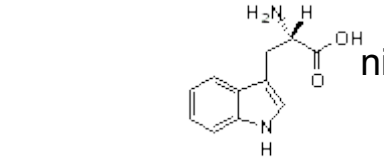
Deep UV Fluorescence Spectra of 52 Compounds

with no baseline subtraction or compensation, Ex=248 nm



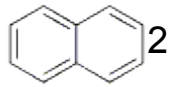
Chemical Differentiability Using Deep UV Excited Fluorescence Alone

A single deep UV laser pulse determines the location of an unknown substance in this chemometric space



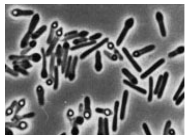
nitrogen based heterocycles **(E)** including tryptophan

3 ring polyaromatic hydrocarbons (PAHs) **(F)**



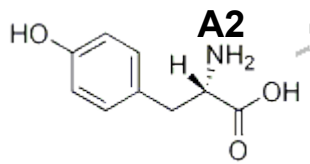
2 ring aromatics **(D)**

vegetative bacterial cells (Gram + and Gram -) with cellular components **(C),**



bacterial spores **(B)**

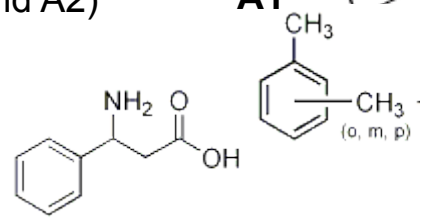
4 ring PAHs **(G)**



A2

1 ring compounds incl. Tyr & Phe (A1 and A2)

A1



(H)

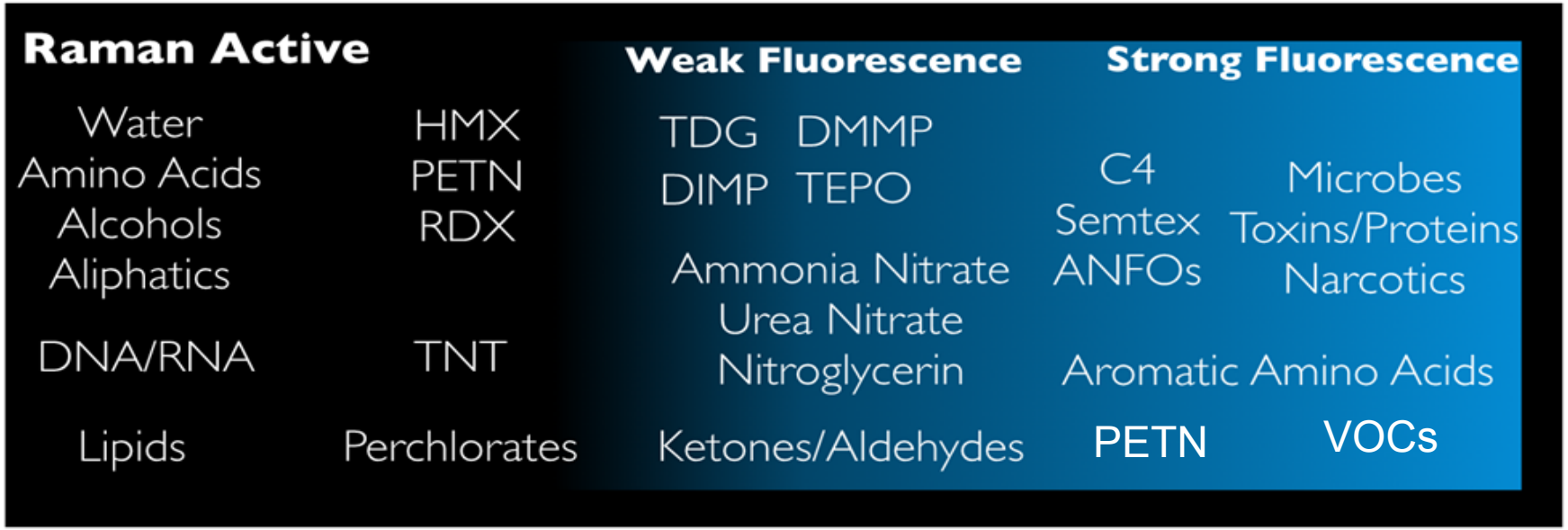
>5 Ring PAHS **(I)**

"background" group **(H)**, consisting of pollens, dust, minerals, and household materials (sugar, flour, corn starch, etc)

PHOTON SYSTEMS

Combining the Sensitivity of Fluorescence & specificity of Raman

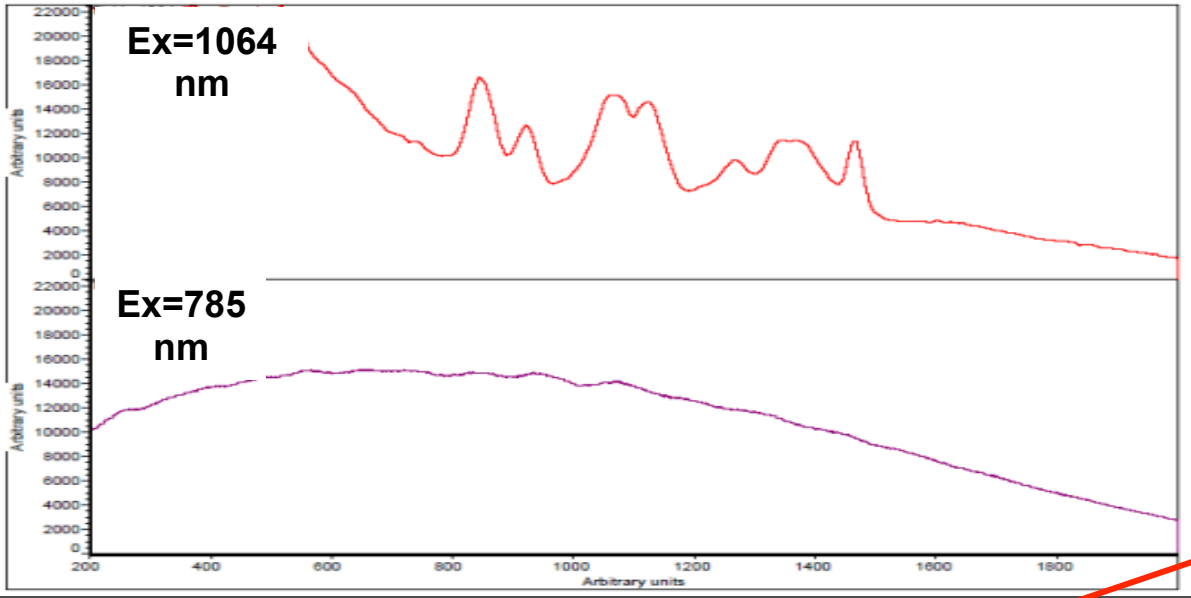
- Fluorescence is the most sensitive method of detection, over 10^6 to 10^8 times more sensitive than Raman, providing longer standoff distances and/or detection at lower concentrations
- Raman provides information about chemical bonds and functional groups, including those that do not fluoresce (aliphatics and simple compounds)
- Fluorescence data provides information about the overall electronic structure of target & substrate components (aromatics, ketones, aldehydes)

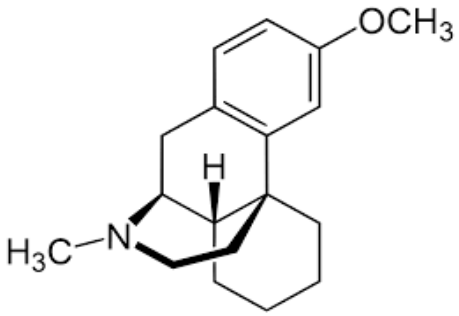


Detection Examples for Pharma Applications

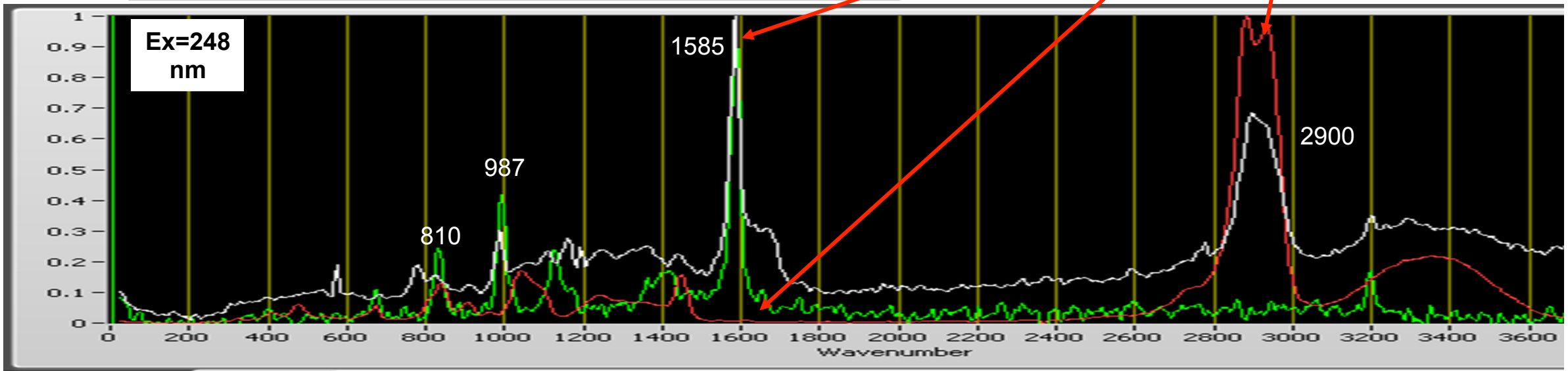
OTC Benylin: dextromethorphan hydrobromide

$C_{18}H_{28}BrNO_2$ Raman spectra





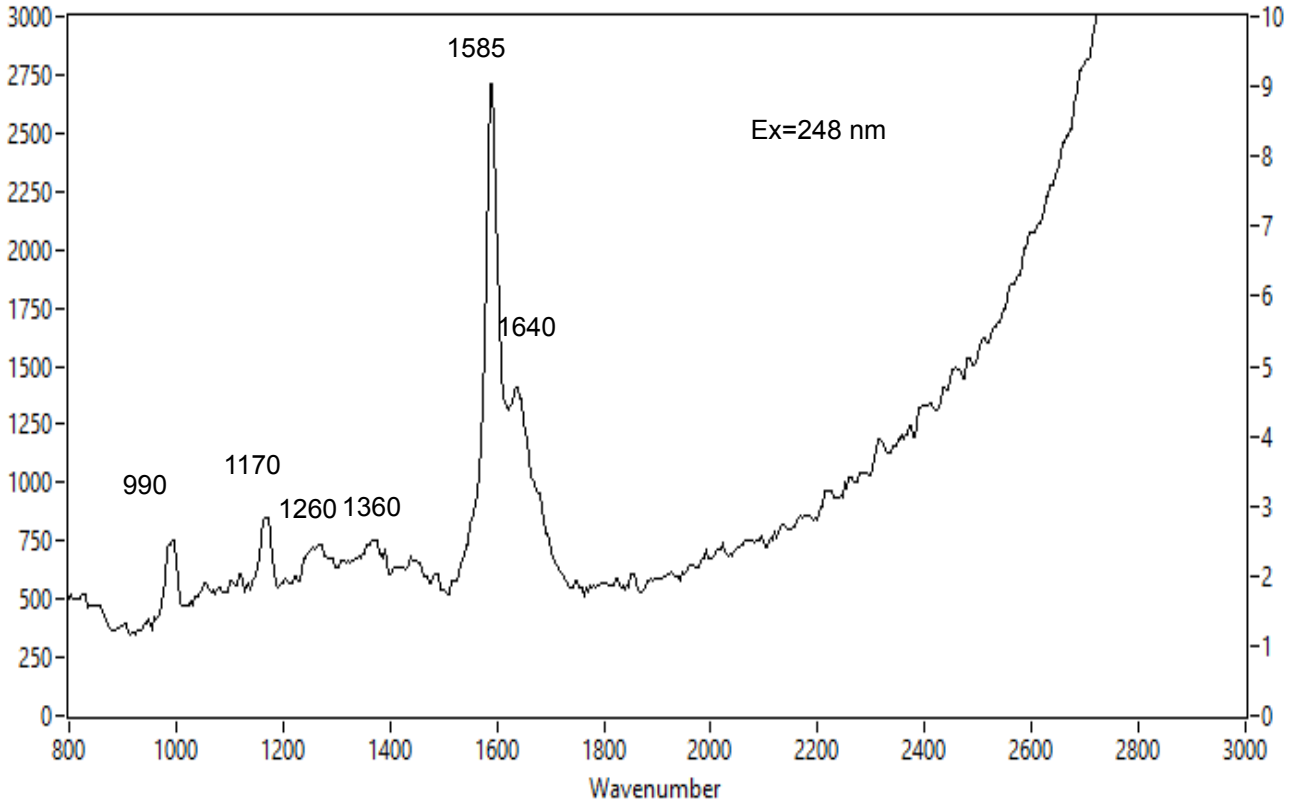
Benylin (white)
Sodium benzoate (green)
Glycerol (red)



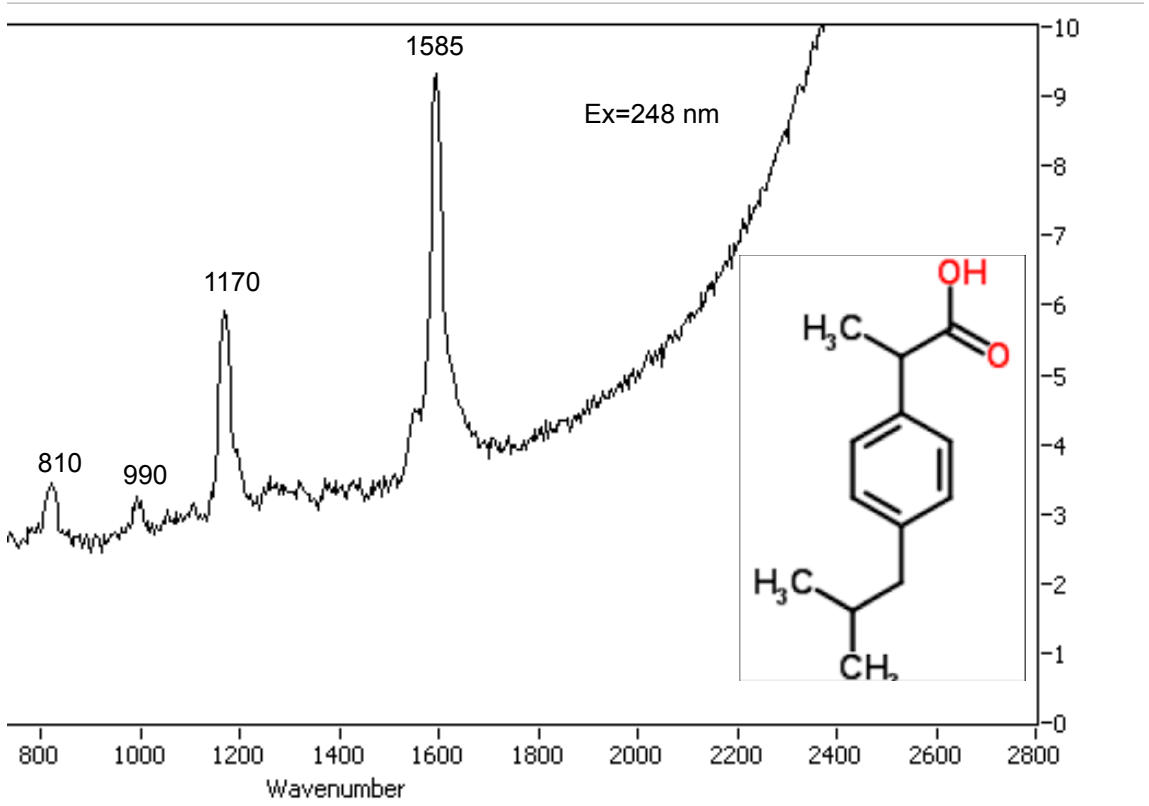
OTC Children's Motrin (ibuprofen)–Bubblegum Flavor

Ex = 248 nm

Children's Motrin-Bubblegum flavor

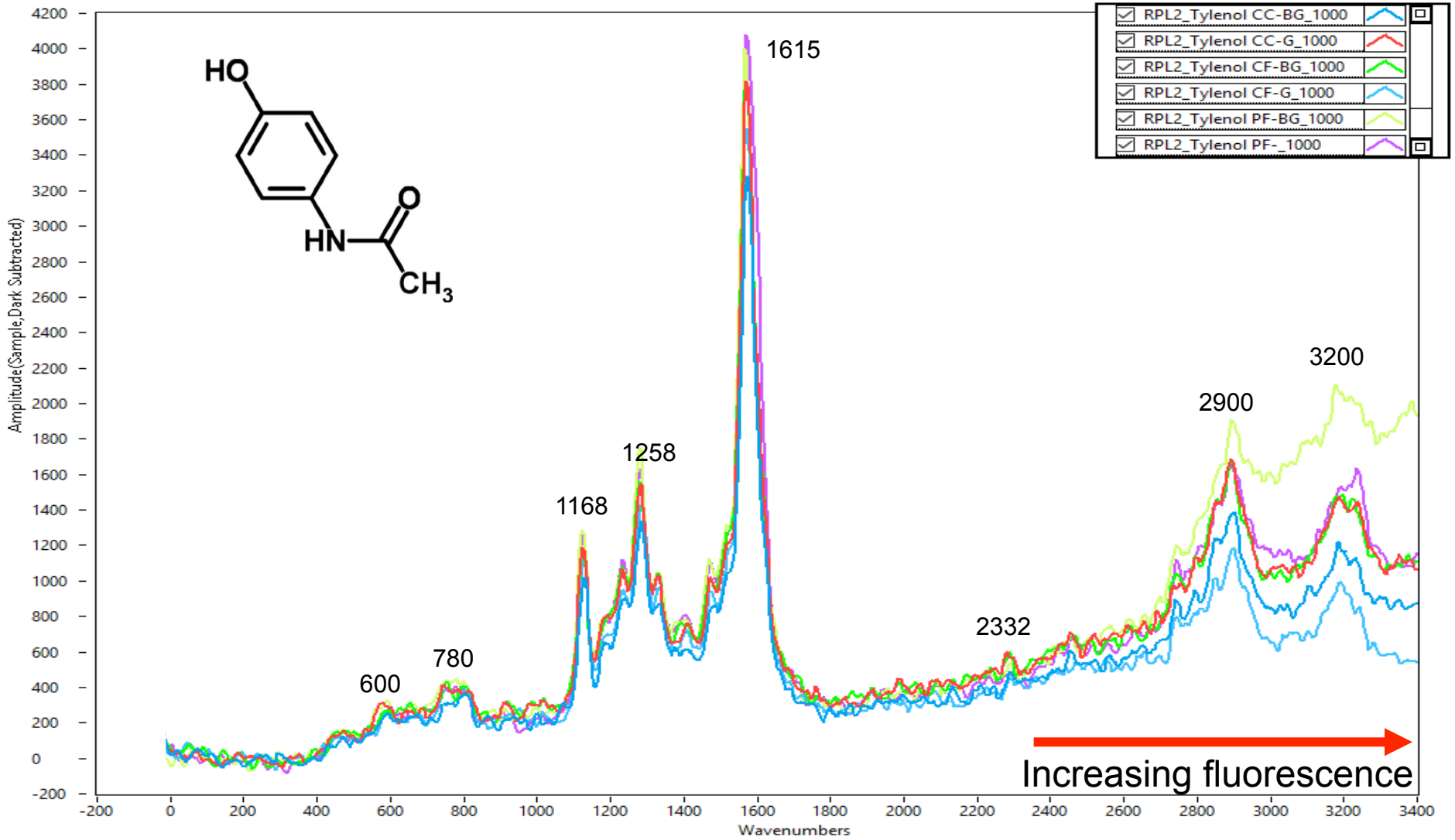


Pure Ibuprofen



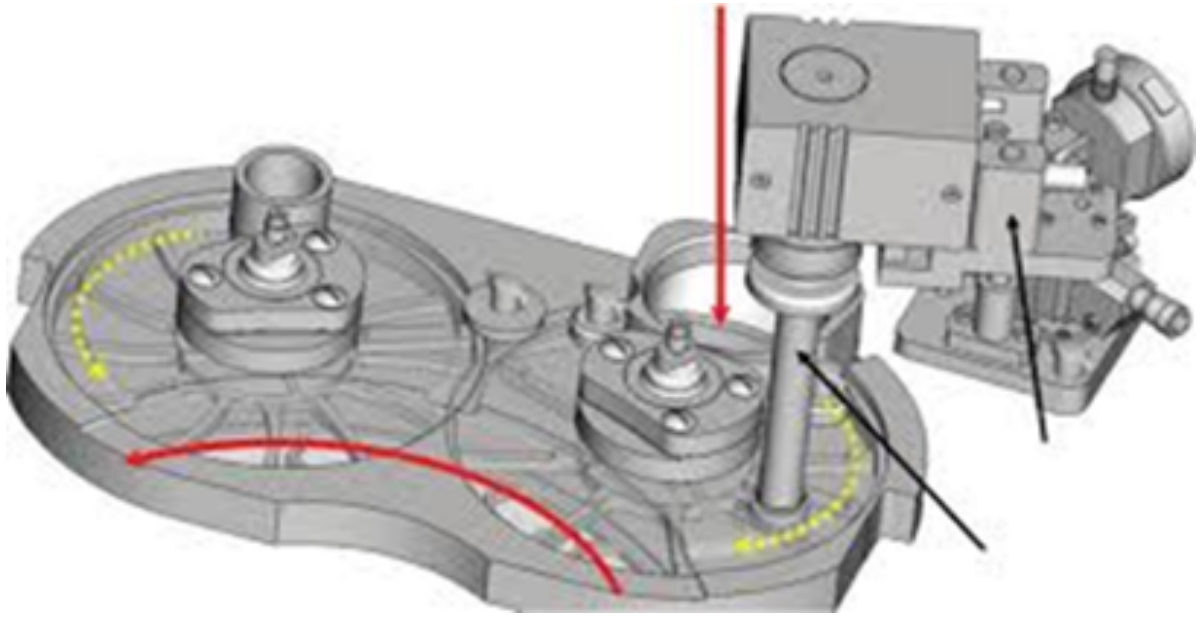
OTC Children's Tylenol (acetaminophen) w Various Flavors

Ex = 248.6 nm Raw results. No baseline compensation.



Detection and control of powder mixtures between 3% and 0.1% using Saccharin as the API (final feed flow)

- Instruments used for this analysis
- Experimental set up
- RPL200 High Resolution fluorescence results
- TraC results
- RPL200 Raman results
- Summary



Detector Choices for Moving Powder

Deep UV application

Fluorescence

- High sensitivity with API
- Typically 5 order magnitude more sensitivity than Raman
- Easy to configure fluorescence detection to optimize sensitivity and specificity
- Meets GMP requirements

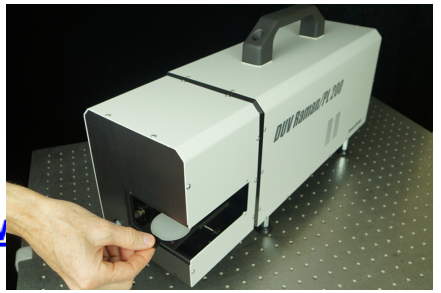
Raman

- High specificity, unique spectral fingerprint
- Complimentary to Fluorescence difference spectral space

- **TraC: Small multi channel Deep UV fluorescence with high sensitivity**

- **TUCS: Multichannel Deep UV Fluorimeter Laser based 248nm**

- **Raman PL 200:** High resolution deep UV Raman & fluorescence instrument



PHOTON SYSTEMS
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Experimental set up

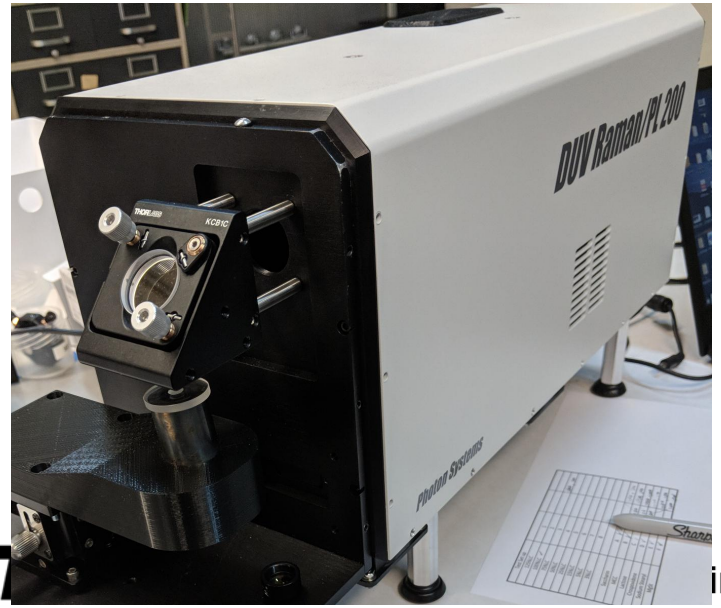
10 grams in each sample	Saccharin target weight	Saccharin Actual weight	MCC Target weight	MCC Actual weight	Lactose target weight	Lactose Actual weight	Crospovidone (Kollidon CL-SF) target weight	Crospovidone (Kollidon CL-SF) Actual weight	Sodium Stearyl Fumarate (PRUV) target weight	Sodium Stearyl Fumarate (PRUV) Actual weight	MgSt target weight	MgSt Actual weight
		3.75%		59.50%		29.75%		4.00%		3.00%		0%
	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
125%LC	0.46825	0.47818	5.85665	5.85654	2.9751	2.99112	0.3	0.31283	0.3	0.30582	0.1	0.10262
100%LC	0.375	0.38685	5.9499	5.96275	2.9751	2.98807	0.3	0.30847	0.3	0.30206	0.1	0.09961
75%LC	0.28125	0.28681	6.04365	6.04035	2.9751	2.99067	0.3	0.30714	0.3	0.29903	0.1	0.10041
50%LC	0.1875	0.19083	6.1374	6.14877	2.9751	2.99162	0.3	0.30631	0.3	0.30099	0.1	0.10305
25%LC	0.09375	0.09519	6.23115	6.23837	2.9751	2.98859	0.3	0.30744	0.3	0.29836	0.1	0.09996
15%LC	0.05625	0.05891	6.26865	6.26588	2.9751	2.98591	0.3	0.30135	0.3	0.29928	0.1	0.90978
5%LC	0.01875	0.01852	6.30615	6.30915	2.9751	3.00769	0.3	0.30434	0.3	0.29931	0.1	0.09949
3%LC	0.01125	0.0119	6.31365	6.31137	2.9751	2.98992	0.3	0.30511	0.3	0.299	0.1	0.09911

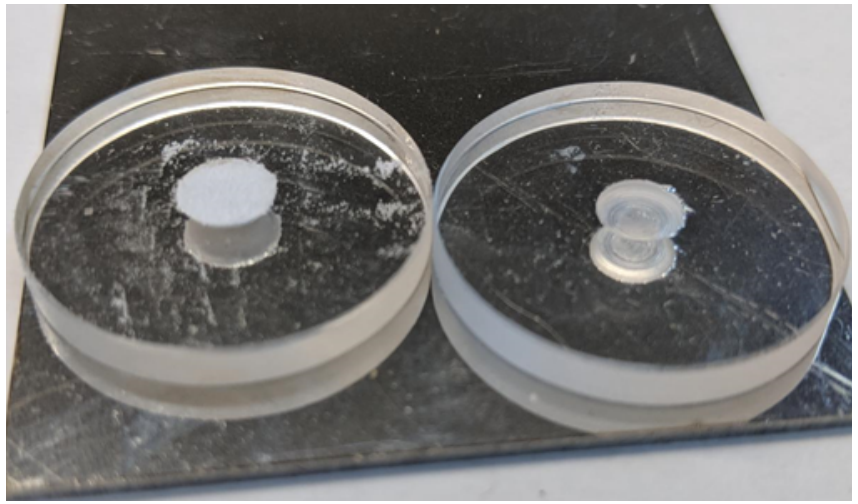
Preparation Fluorescence

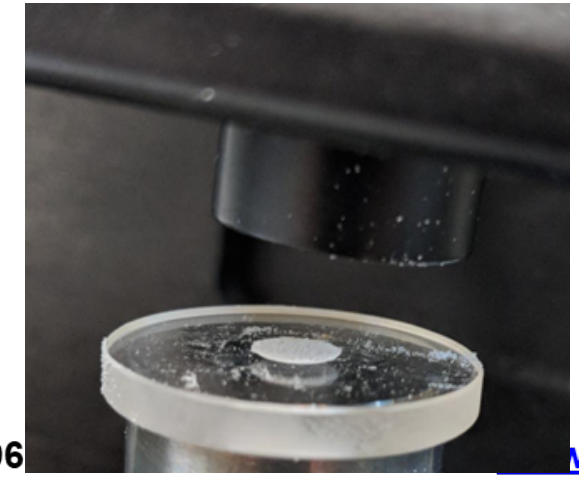
- Samples were loaded into a stainless steel apparatus containing 9 cells ½ inch in diameter covered by a 5cmx5cm Quartz window.
- Each sample was loaded in the rear with a funnel and each cell was plugged with a screw to avoid cross contamination.
- A-F API and Excipients
- 1-8 varying prepared concentration samples of Saccharin.

Preparation Raman

- Due to the quarts cover window fluorescence interference we used direct view of the sample with no cover.
- Holes are 5mm diameter by 3mm deep.





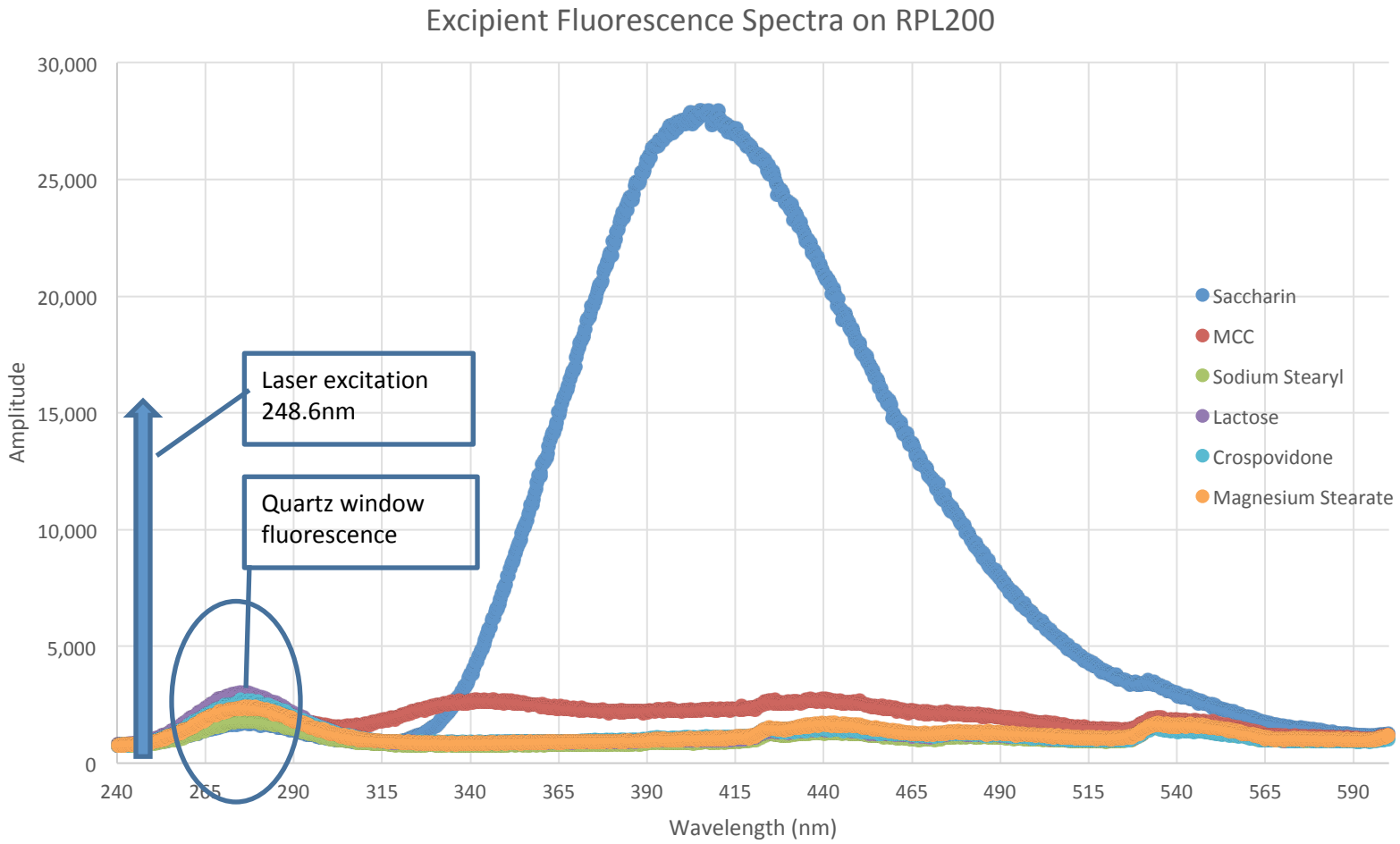


RPL200 Excipients and API Fluorescence

High Resolution

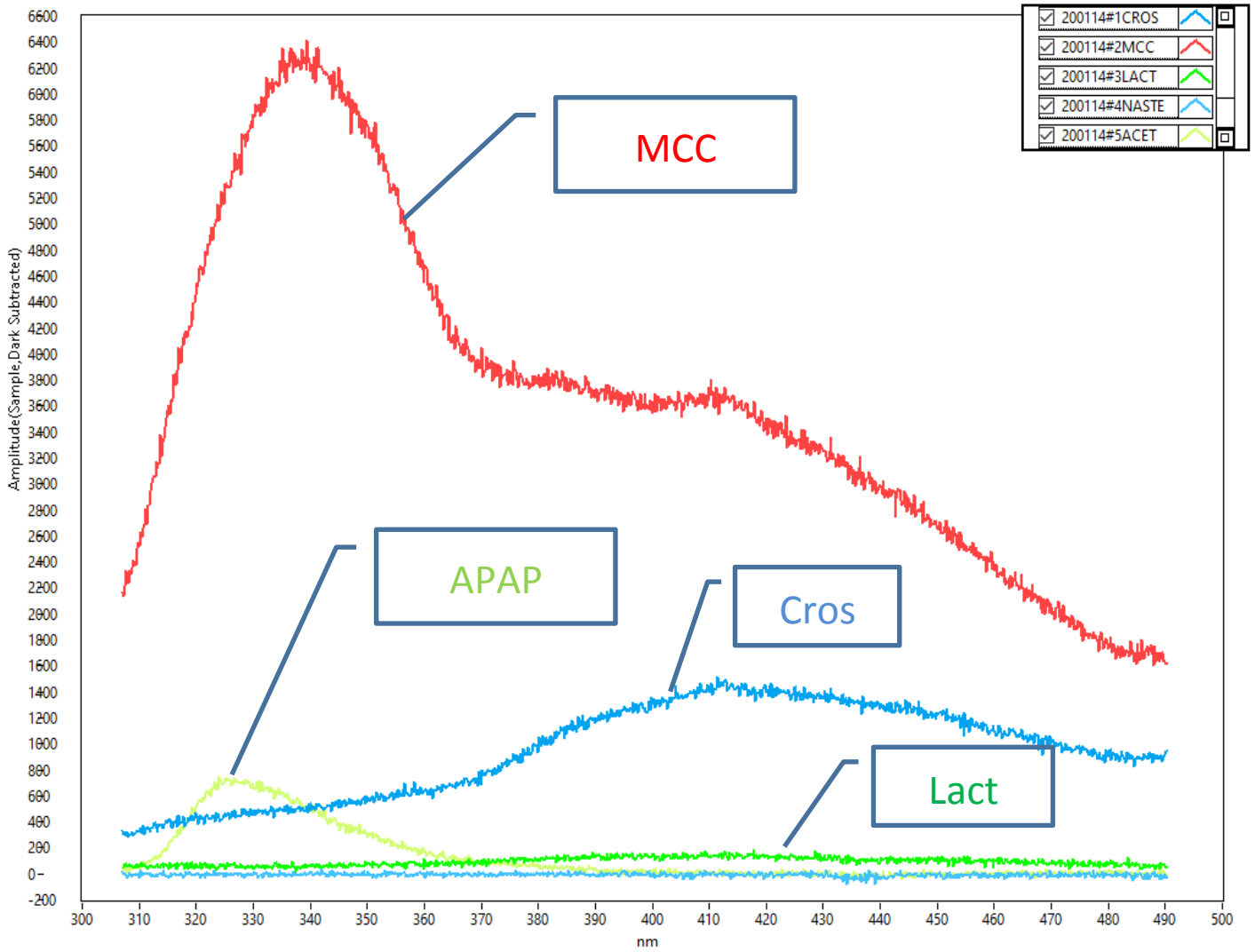
RPL200

- RPL setup with 30mm objective lens and with 1 pulse at 40Hz.
- Saccharin peak shows very intense fluorescence with peak at 405nm
- Spectra at 280nm is fluorescence of the Quarts cover plate. It is important to chose the correct site window material.
- The excipients remain about an order of magnitude lower fluorescence.



High resolution Fluorescence Excipients and API

(spectra taken at 1pulse).



- Note most excipients have broad fluorescence emission and APIs are typically narrower (FWHM).
- We see here that unfortunately for this application the API (APAP) is significantly lower in fluorescence emission response and is not substantially different in spectral location than MCC.
- We would predict that fluorescence would not be an adequate technique to differentiate or provide concentration information for APAP.

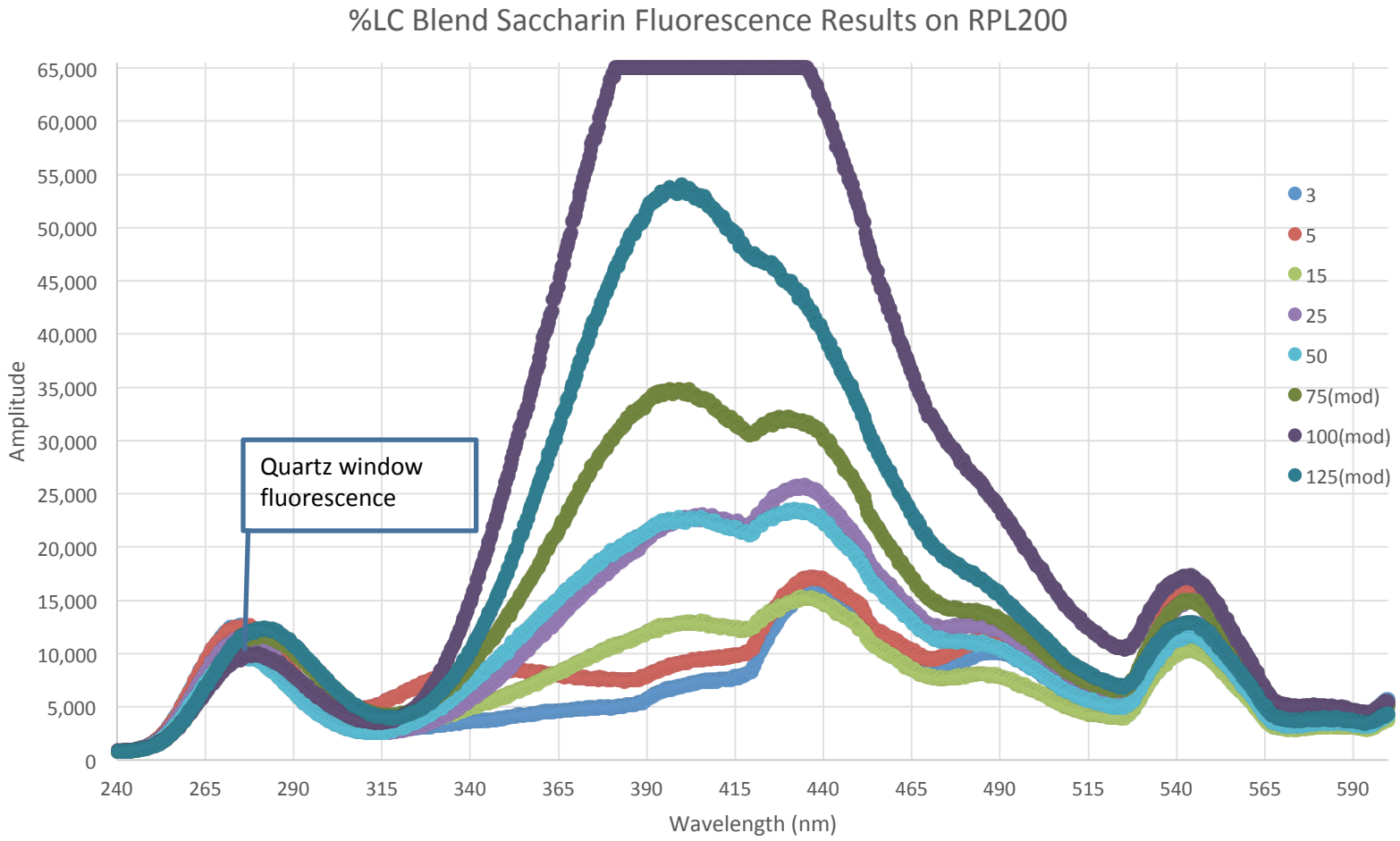
RPL200 Saccharin Blend Fluorescence

High Resolution

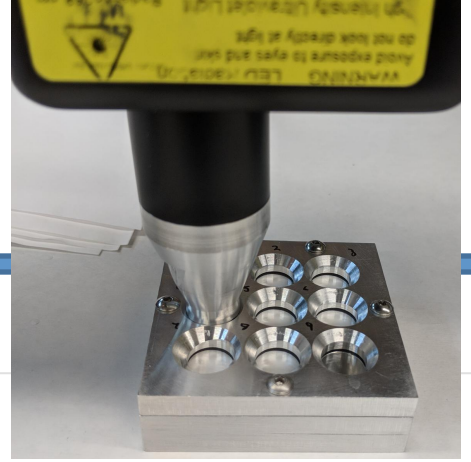
RPL200 Fluorescence

- RPL setup with 30mm objective lens and with 1 pulse at 40Hz.
- Beam diameter ~50um

Due to the small beam relative to the API and excipients particle size, some inconsistencies in the ratios are observed.

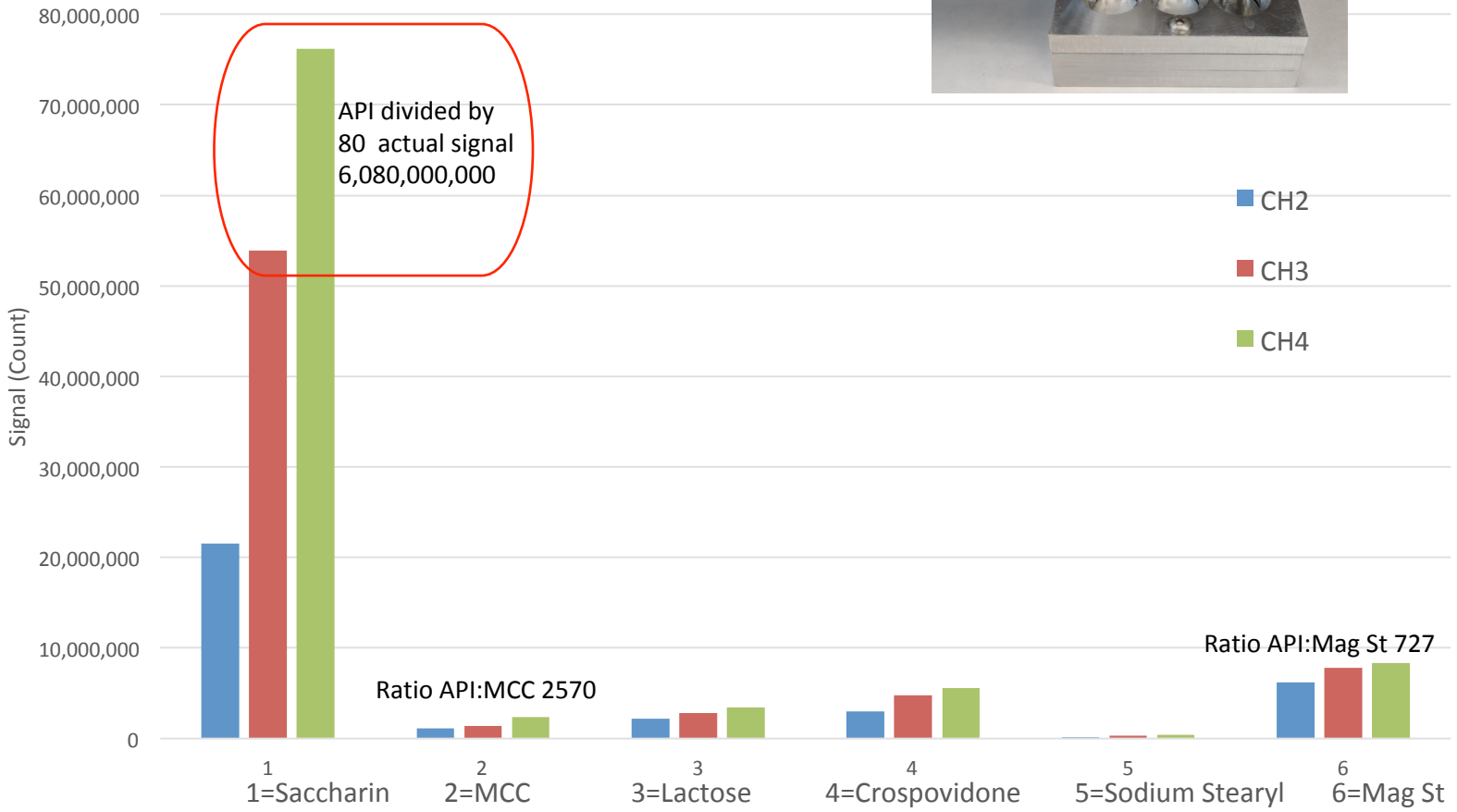


TraC Excipients & Saccharin fluorescence response



TraC (6mm Dia, 5msec pulse one pulse per data point)

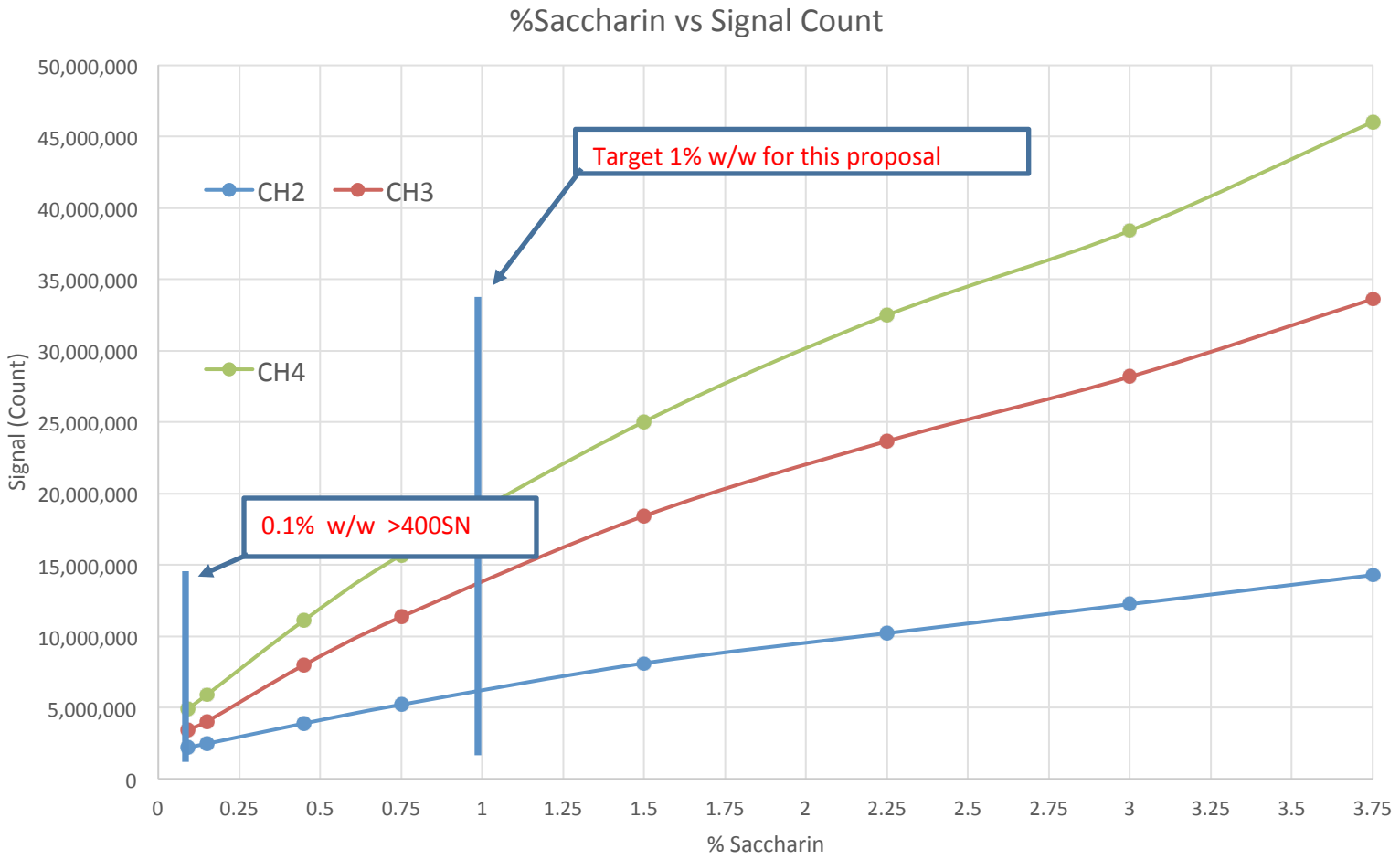
- TraC-400nm contains 268nm excitation LED 3 channels with band pass filters of 389, 432 and 475nm.
- Note again the 389nm filter consistent with the High Res results.
- The UV response for Saccharin is over 750 times that of the other excipients.
- Signal to background is > 2000



TraC saccharin dilutions fluorescence response

TraC

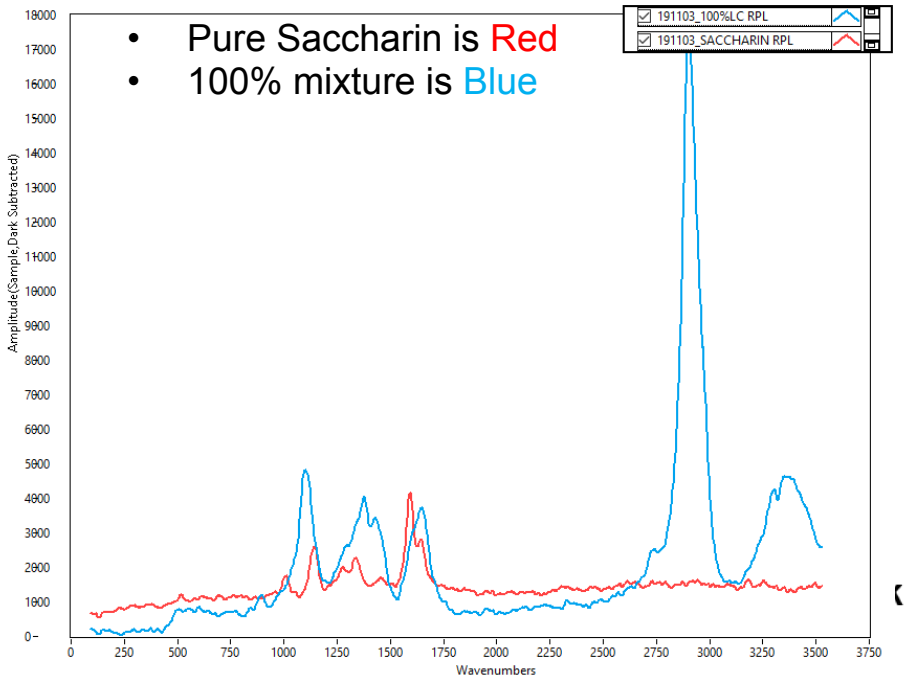
- The signal output from Saccharin was saturating the photodiode, so we had to decrease the current from 0.8A to 0.2A and maintain the pulse width at 200us (max of 5000us). This means we have more than 2+ orders of magnitude for weaker fluorescing APIs.
- At 0.1% API we would estimate SN at >400
- We would estimate the LOD with SN of 3 to be ~0.04% for Saccharin.



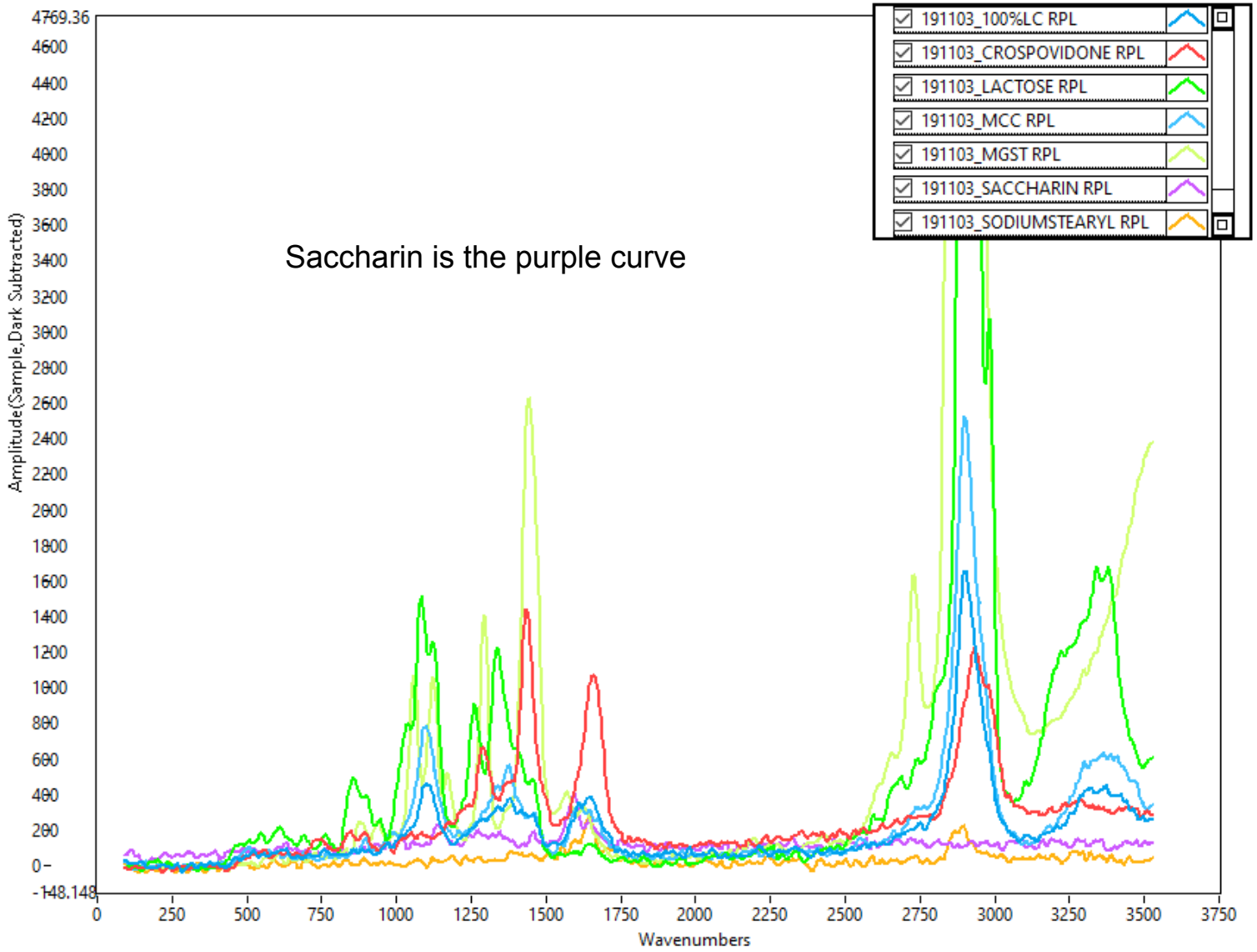
RPL 200 Raman results

Raman Excipients and Saccharin

Saccharin has a very low Raman signature compared to the other excipients. It does not seem likely that Raman could be used in this case as an online sensor for Saccharin.



- Pure Saccharin is Red
- 100% mixture is Blue



Saccharin is the purple curve

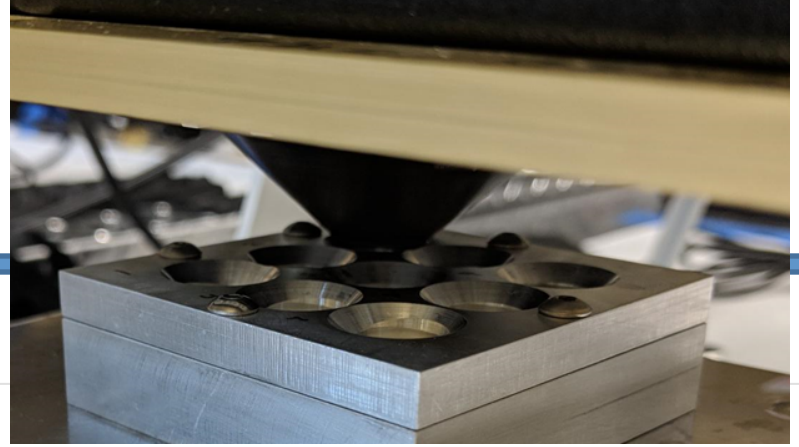
Detection and control of powder mixtures

3% to >0.1% using Saccharin as the API
(Fluorescence and Raman results)

- Deep UV fluorescence easily meets the requirements for Saccharin.
- With such detection margin, using either a fiber bundle of a light pipe will simplify the machine integration.
- These nominal excipients offer little interference with fluorescence.
- As these are dry powders there will be no dynamic fluorescence characteristics as seen with the liquid/solvent printing API.
- Triggering for system integration should not be a problem (200us-5ms) pulse time.

Questions ?

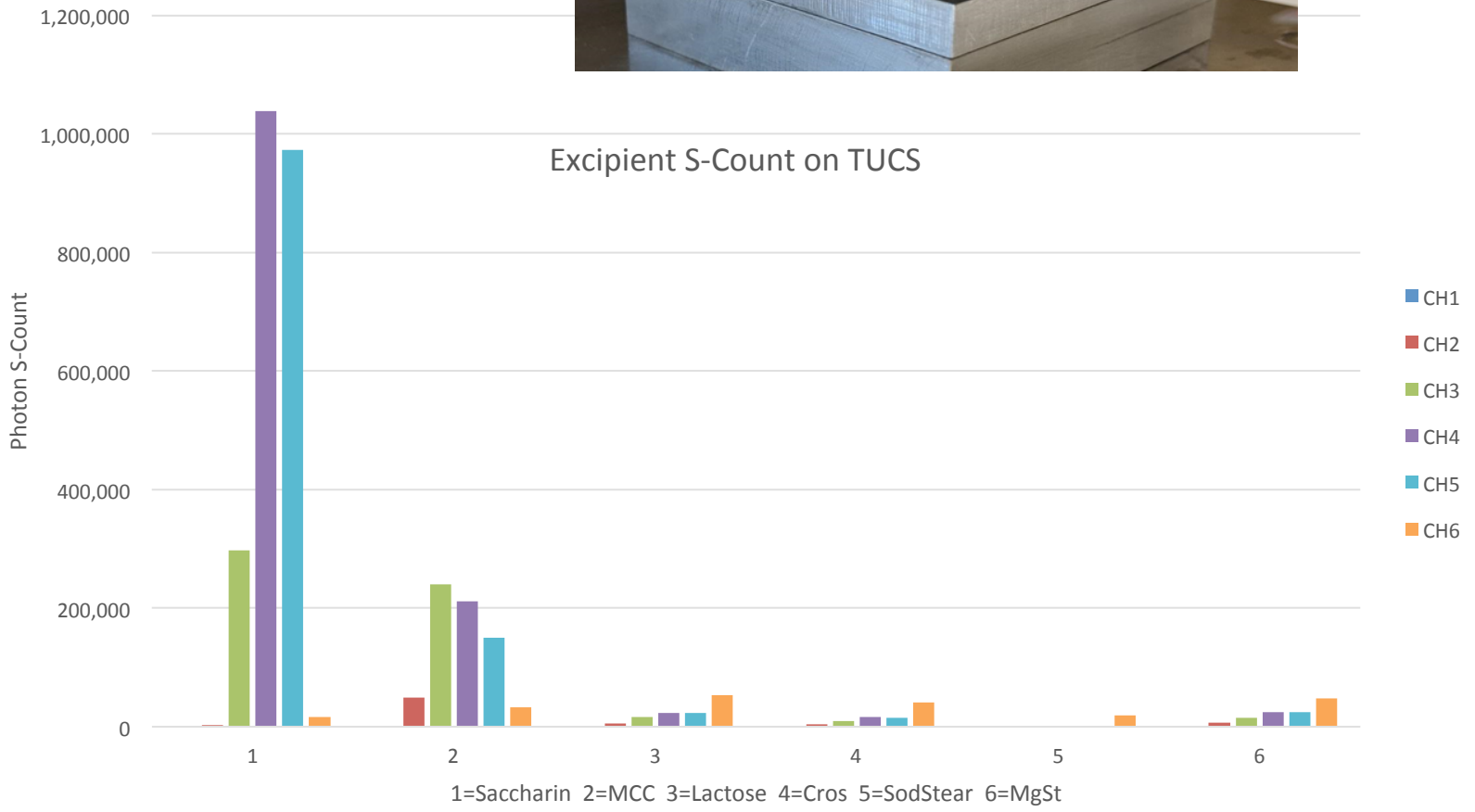
TUCS API & Excipients Fluorescence



TUCS

- Excitation at 248.6nm (laser)
- Beam diameter ~3mm Ring
- 6 discrete emission filters
- PMT detector, single pulse~40us

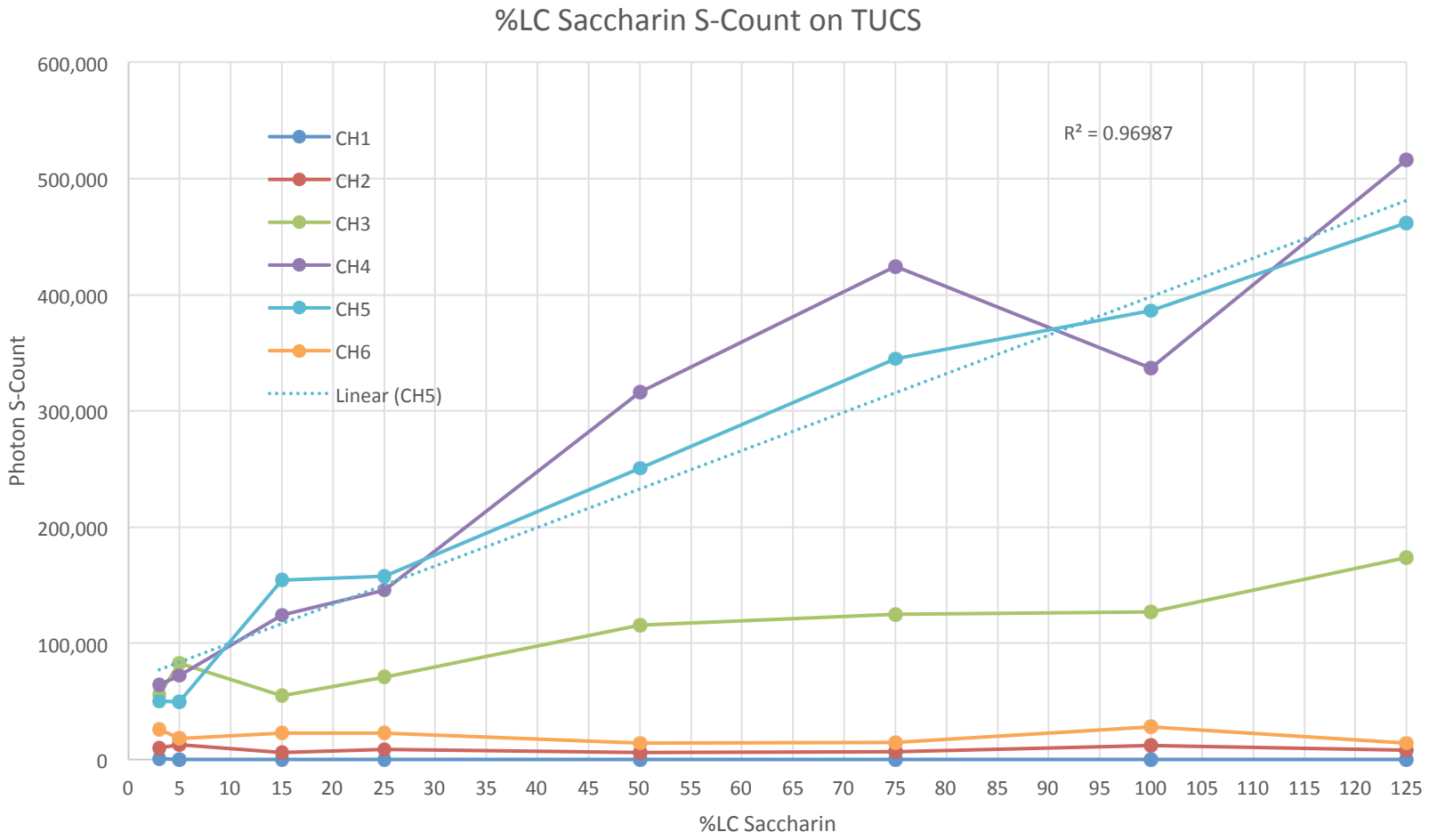
- Peak fluorescence for Saccharin in filter 365nm and 387nm. They have different bandwidth but are consistent with the High Res data
- Again the fluorescence results indicate much higher signal values for Saccharin than the Excipients.



Saccharin Blend results with TUCS

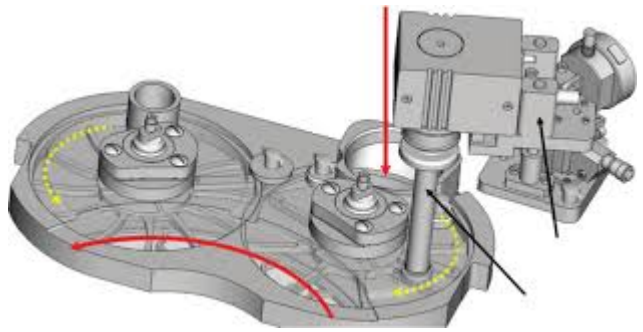
TUCS

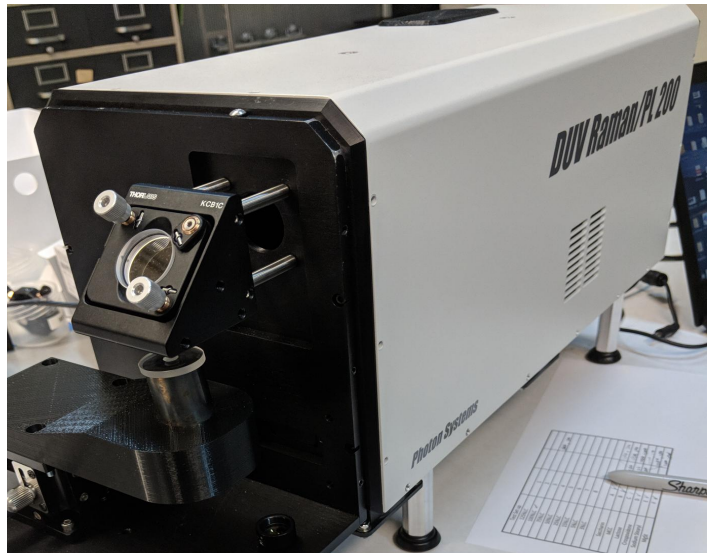
- Plotted here are the 6 fluorescence channels vs Saccharin concentration
- Due to the strong fluorescence we observe a good representation of the Saccharin concentration down to 0.1% and beyond.



Summary Report APAP and excipients

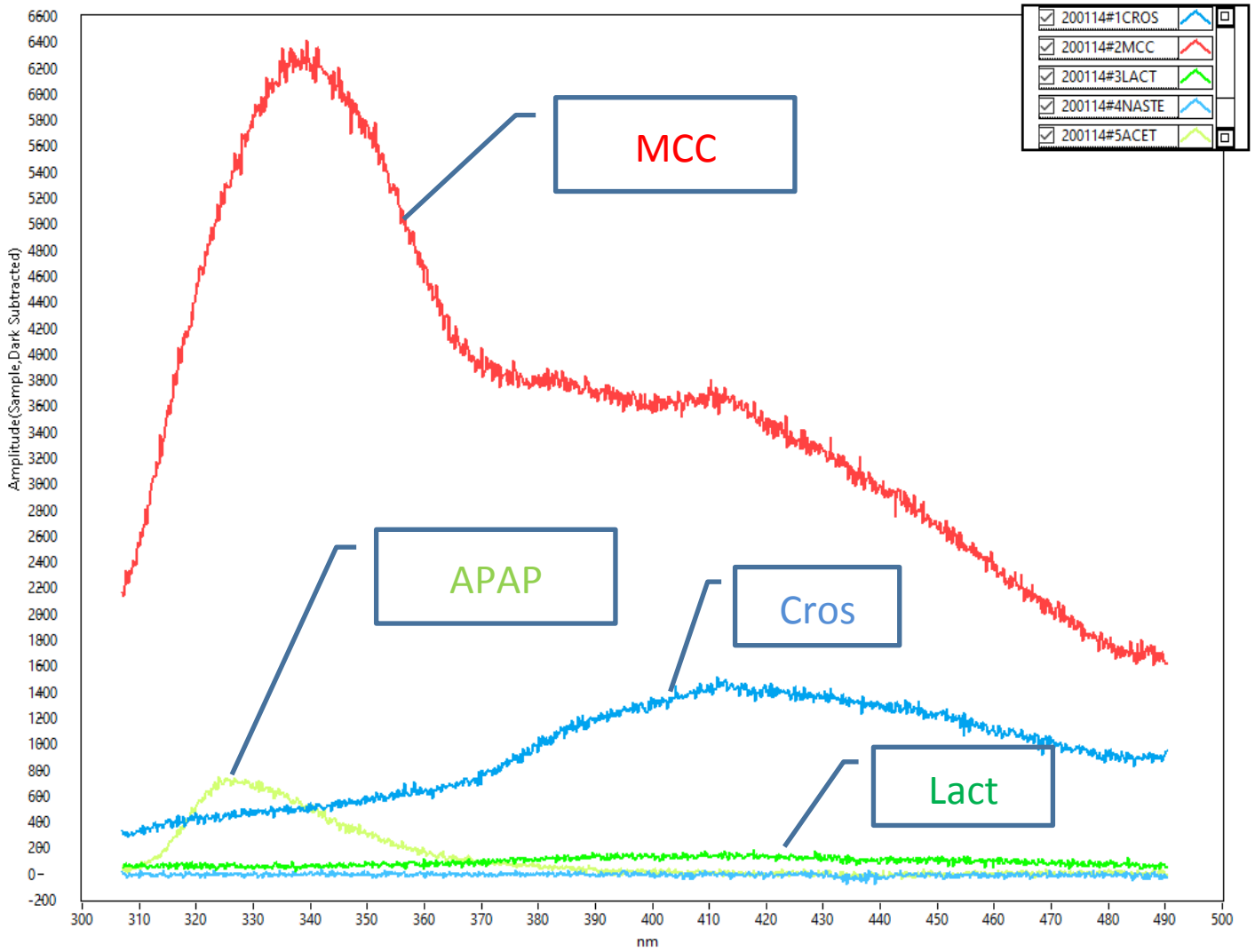
- Lilly provided neat samples of :
  - Acetaminophen (API)(APAP), Croscarmellose, Sodium Stearyl, Lactose Monohydrate, MCC PH102
  - Mixtures; 1.3%, 1.1%,1%, 0.9%, 0.7%
- Photon Systems tested all samples to determined if suitable for high potency low concentration w/w powder online final feed flow concentration analysis;
  - High resolution fluorescence (spectral and relative intensity) response using Deep UV excitation (248nm)
  - Deep UV Raman spectral analysis (spectral and intensity) response
- Summary





High resolution Fluorescence Excipients and API

(spectra taken at 1pulse).



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- We see here that unfortunately for this application the API (APAP) is significantly lower in fluorescence emission response and is not substantially different in spectral location than MCC.
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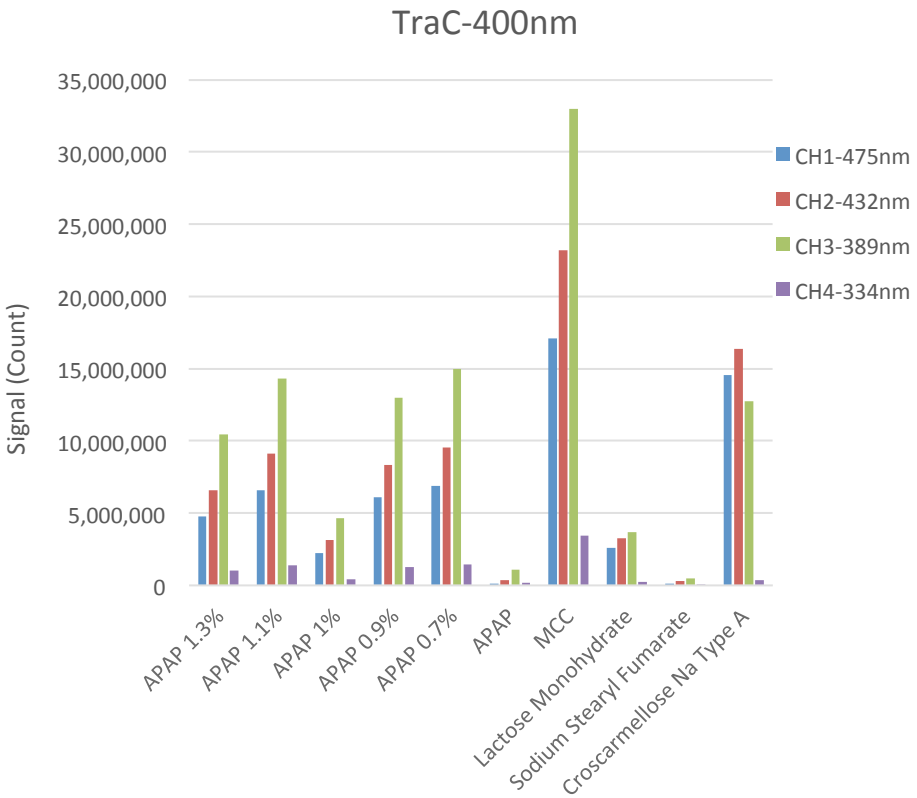
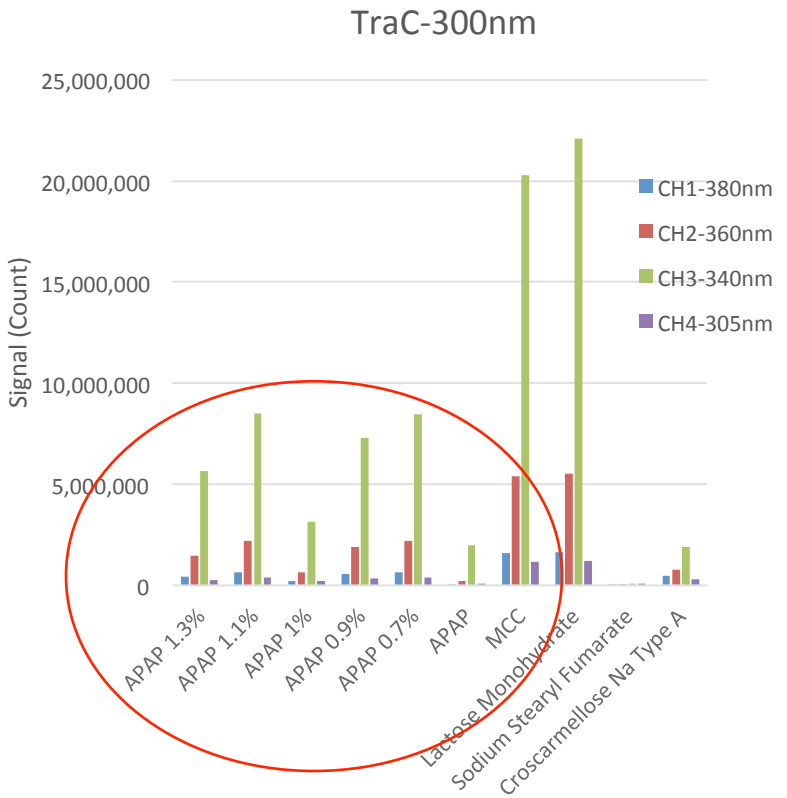
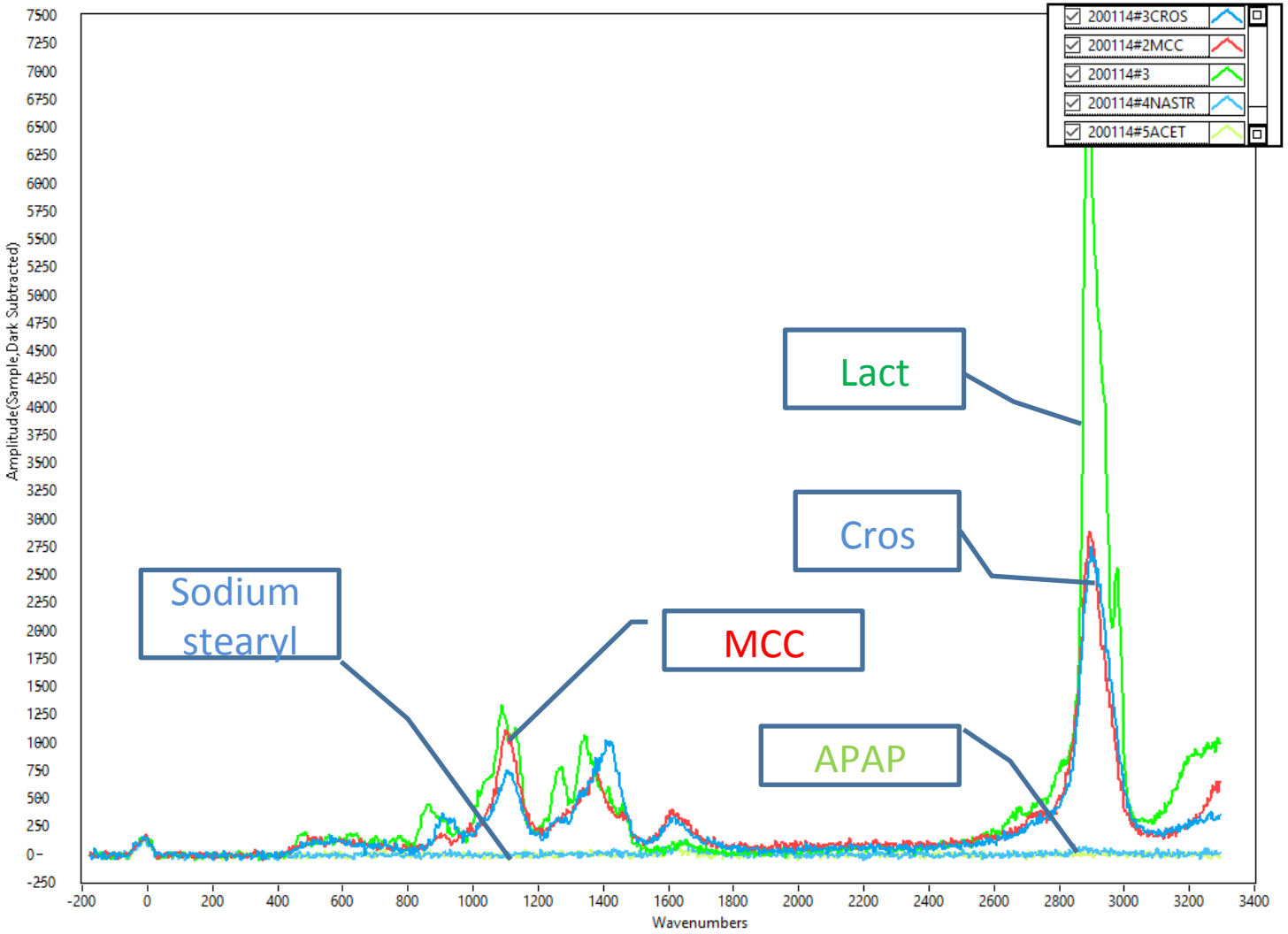
Multi fluorescence emission channel instrument TraC-xxxnm





Looking at both versions of the TraC (300nm and 400nm) we see no concentration trend in the APAP dilution/concentration samples. Recalling the peak emission wavelength for APAP is ~325nm we would look at channel 4 on the TraC-400 device and at 340nm band(channel 3) on the TraC-300 instrument. You see on the TraC300 the Green (340nm) band is indeed highest but that is a result of the combined fluorescence of both the APAP and MCC with no way to differentiate the contribution of each.

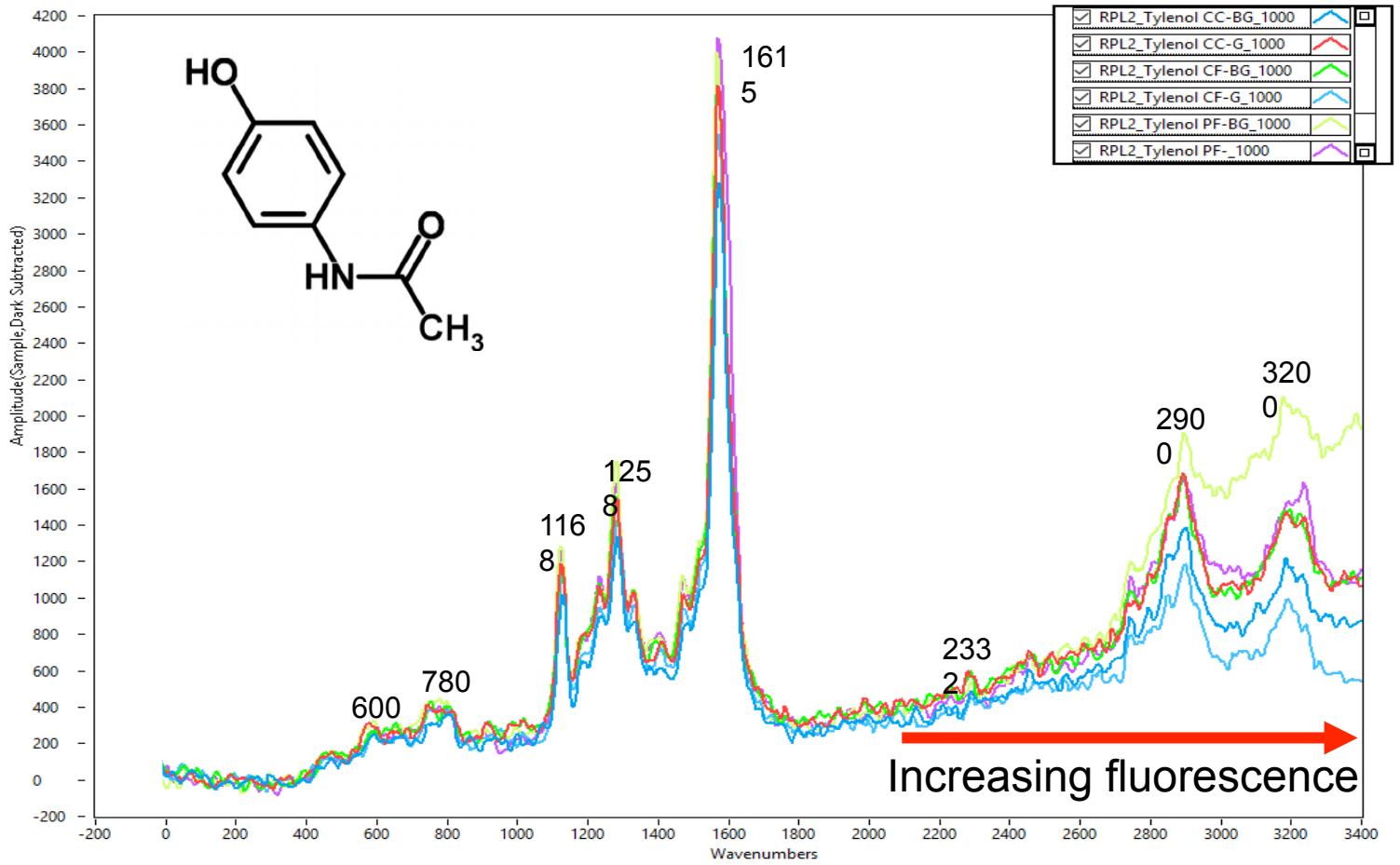
Raman spectra for excipients and API



- The Raman data was taken with the same settings, 100Pulses therefore the intensity and spectral data are relative for all materials
- Note the Raman spectra of APAP is very week.
- Conclusion is that Deep UV Raman spectra of APAP will not provide online concentration sensitivity required for this application

OTC Children's Tylenol (acetaminophen) w Various Flavors

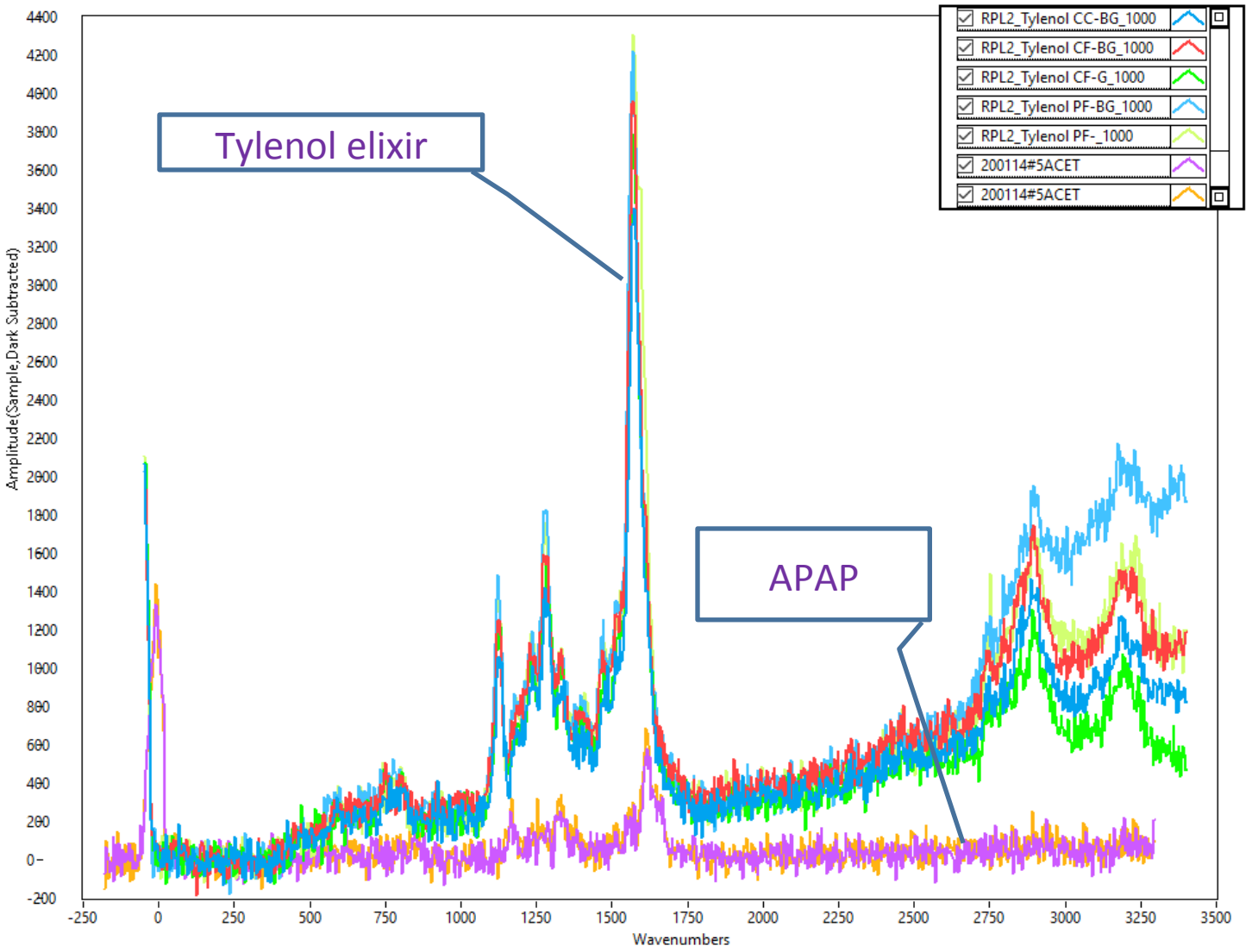
Ex = 248.6 nm Raw results. No baseline compensation.



This is previous data we had taken on Tylenol (acetaminophen) showing very robust Raman signals of different flavors of a children liquid elixir.

At cm-1 increasing above 2000 you begin to see the unique fluorescence of the excipients.

Comparison of previous data with the pure APAP powder from lilly.



- Shown here is a comparison of the liquid Tylenol and the Lilly powder.
- Besides the obvious missing fluorescence excipient contribution in the liquid elixir we also see very low signal levels of the Pure Powder. Not sure why?

Summary for APAP Raman and Fluorescence

Acetaminophen has relatively low Raman and fluorescence emission intensity, well below, in both cases, that of the excipients. In this case neither of these Deep UV spectroscopic techniques are able to provide quantitative concentration information useful in the range <2% w/w.