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Applied Translational Medicine

The Analysis of Isotopically Labeled Propylene Glycol in eCigarettes

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Presentation flow

- Celerion patent for testing eCigarettes
  - Provisional patent filed September 9, 2013
  - Final patent filed September 9, 2014 (patent pending)
- Chemistry 101
  - Stable isotopes
  - LC-MS/MS
- Describe a bioanalytical plasma assay for propylene glycol (PG) using LC-MS/MS
- APPLIED SCIENCE
  - The power of stable isotopes in clinical studies – $^{13}C_3$-PG
  - Design of an eCigarette pilot study using $^{13}C_3$-PG
    - Results
    - Conclusions

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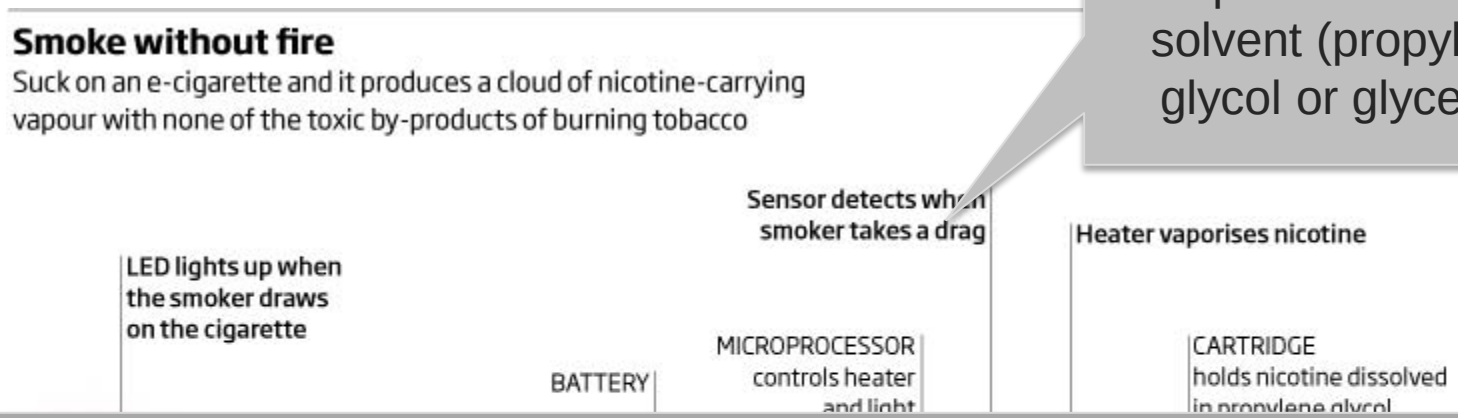
Celerion's Patent for Testing e-Cigarettes

E-Cigarettes vaporize liquid nicotine in solvent (propylene glycol or glycerol)



Problem: Solvents used are ubiquitous. Need to distinguish how much of this constituent get into the blood from the e-Cig, rather than the environment.

Patented Solution: Replace the solvent with a solvent that was made with stable isotopes (mass is greater)!! Use mass spectrometry to distinguish between endogenous levels of the constituents and those that came from the e-Cig

Chemistry 101 - Stable Isotopes

Atom	Identification	# Protons	# Neutrons	AMU
Hydrogen	Hydrogen	1	0	1
	Deuterium*	1	1	2
	Tritium**	1	2	3
Carbon	Carbon	6	6	12
	C13*	6	7	13
	C14**	6	8	14

> 99.99% of hydrogen on earth has an AMU = 1.
> 99.9% of carbon on earth has an AMU = 12.
An isotope of an atom has an atypical AMU.

* **stable** isotope

** unstable isotop**e = radioactive**

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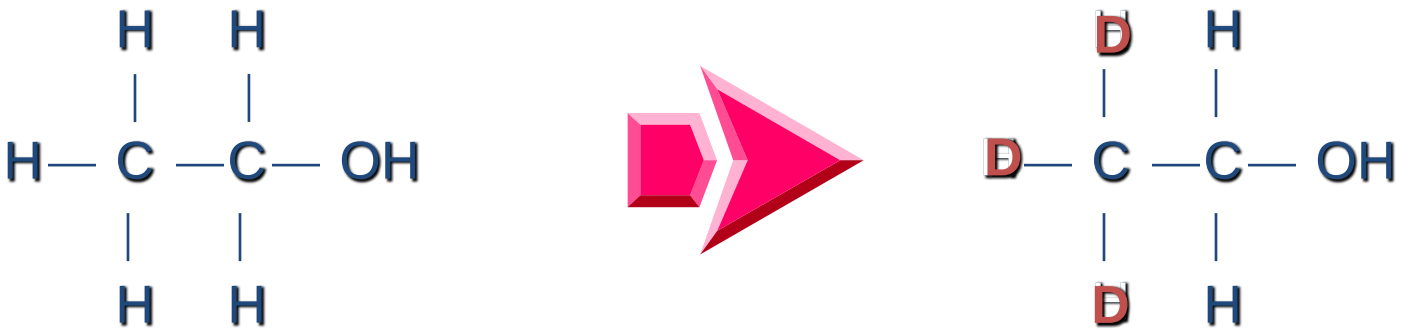
Chemistry 101 - EXAMPLE - Stable Label Drug



- Physical chemical properties d_0-ethanol = d_3-ethanol
- The only instrument/test that can distinguish between d_0-ethanol and d_3-ethanol is a mass spectrometer

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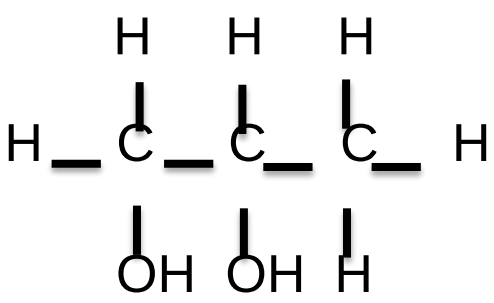
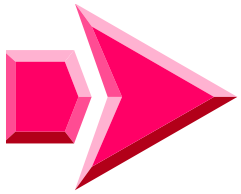
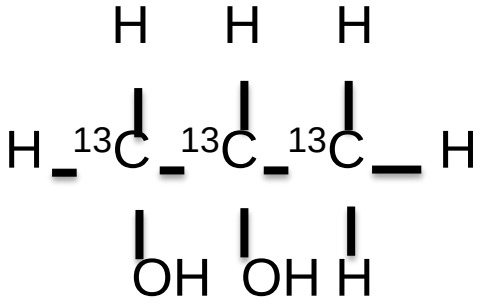
Chemistry 101 - Stable Isotopes of Propylene Glycol (PG)

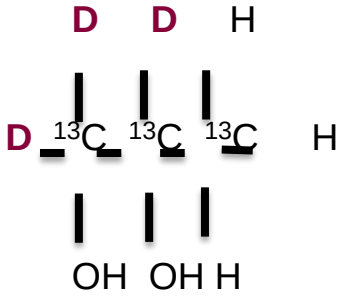


$C_3H_8O_2$
Atomic mass = 76





$^{13}C_3H_8O_2$
Atomic mass = 79
Carbon Heavy PG



$^{13}C_3H_5D_3O_2$
Atomic mass = 82
Analytical Internal Standard

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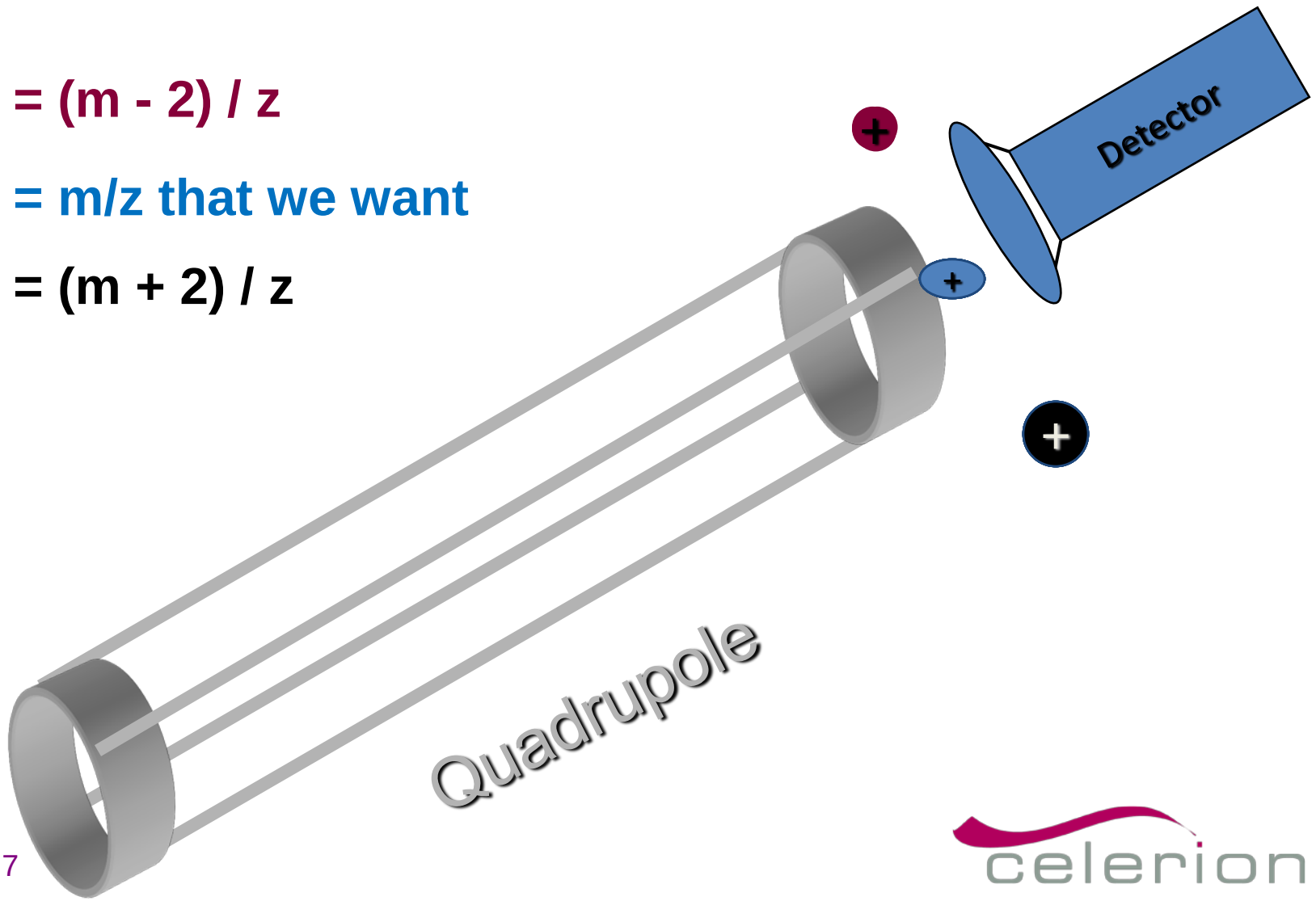
Chemistry 101 - Mass Spectometer in Action

= (m - 2) / z

= m/z that we want

= (m + 2) / z



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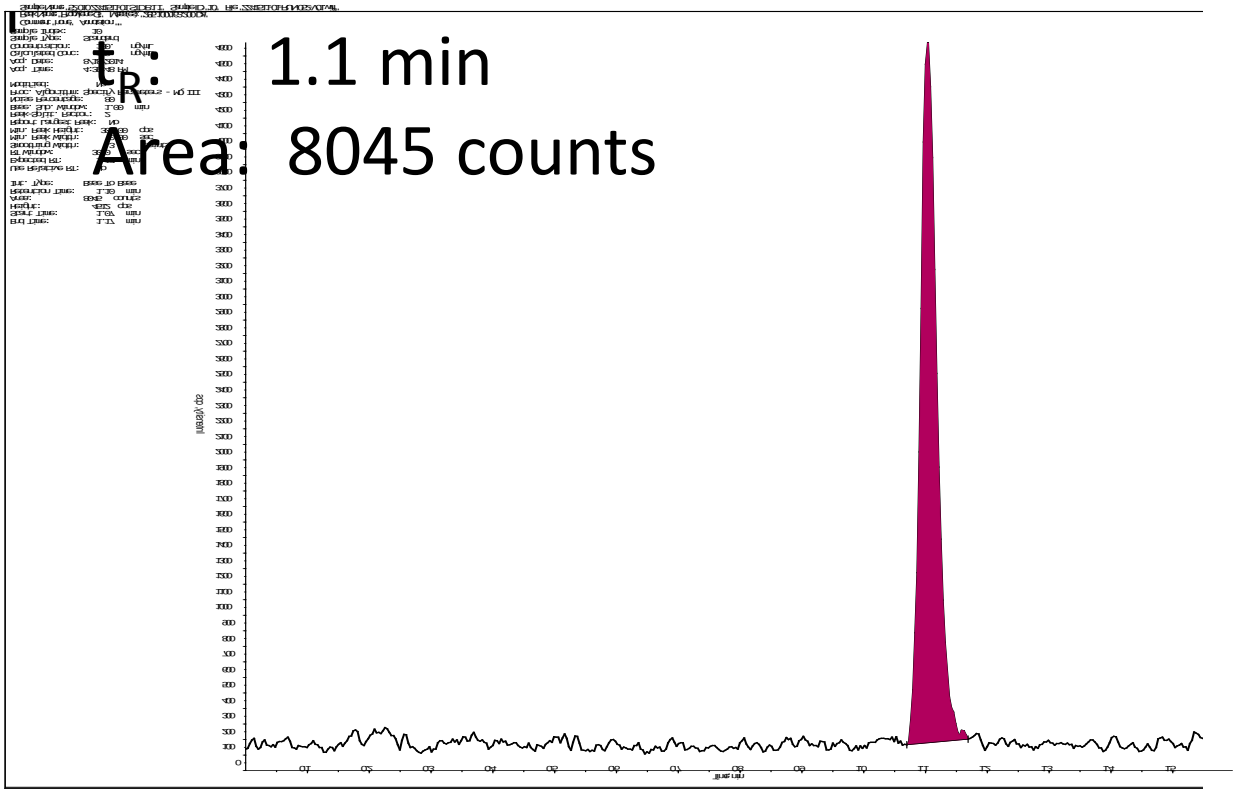
Method Summary Measuring PG in Human Plasma

- Sample
  - 0.05 mL plasma
  - Add Analytical Internal Standard ($^{13}C_3{}^2H_3$-PG)
- Derivatization (to add some mass for LC-MS/MS)
- Liquid-liquid extraction of derivatives
- Reversed-phase gradient UHPLC separation
- Detection with AB Sciex API 4000™ tandem mass spectrometer (ESI positive mode MRM)

PG LLOQ - 100 ng/mL in Ultrapure Water



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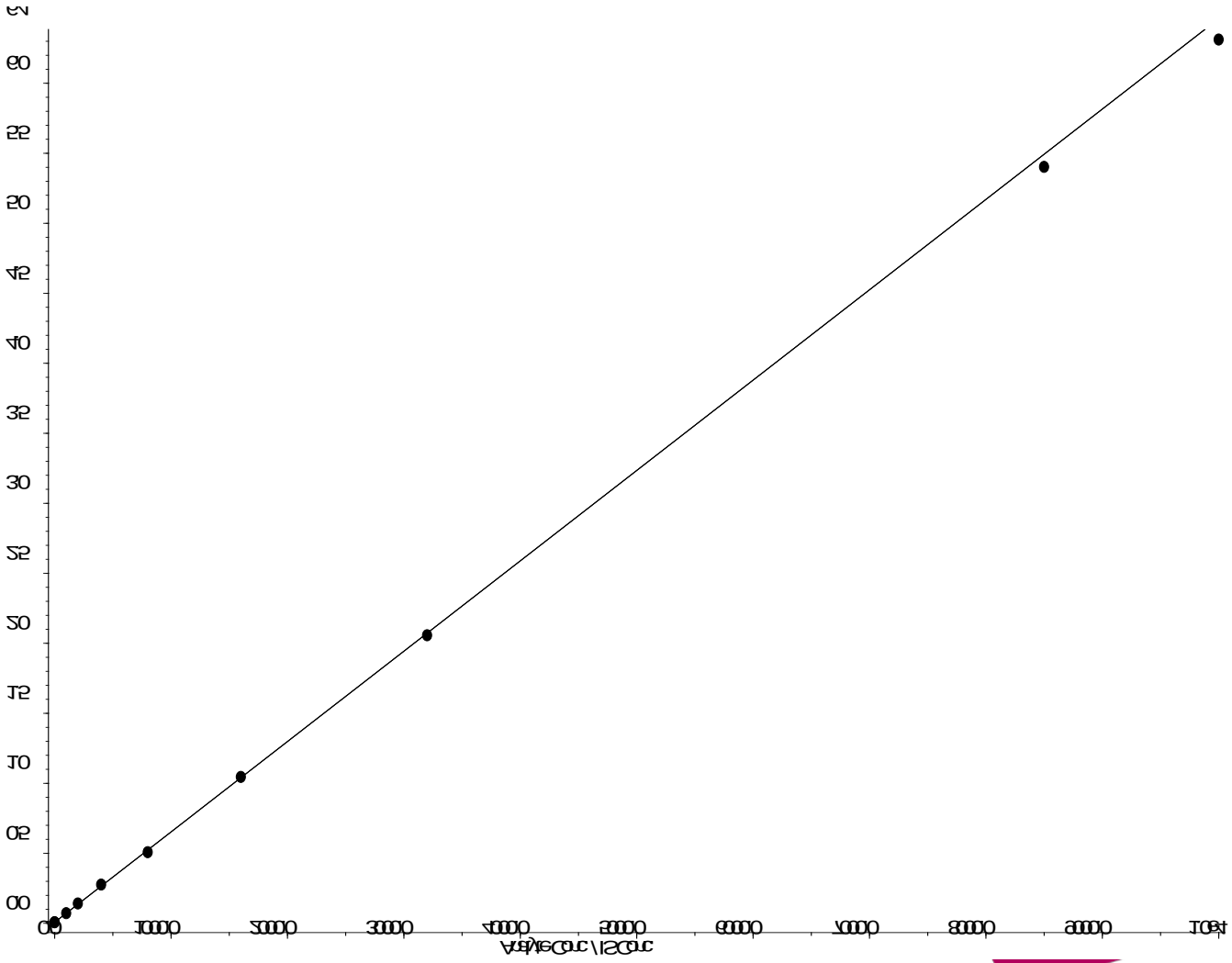
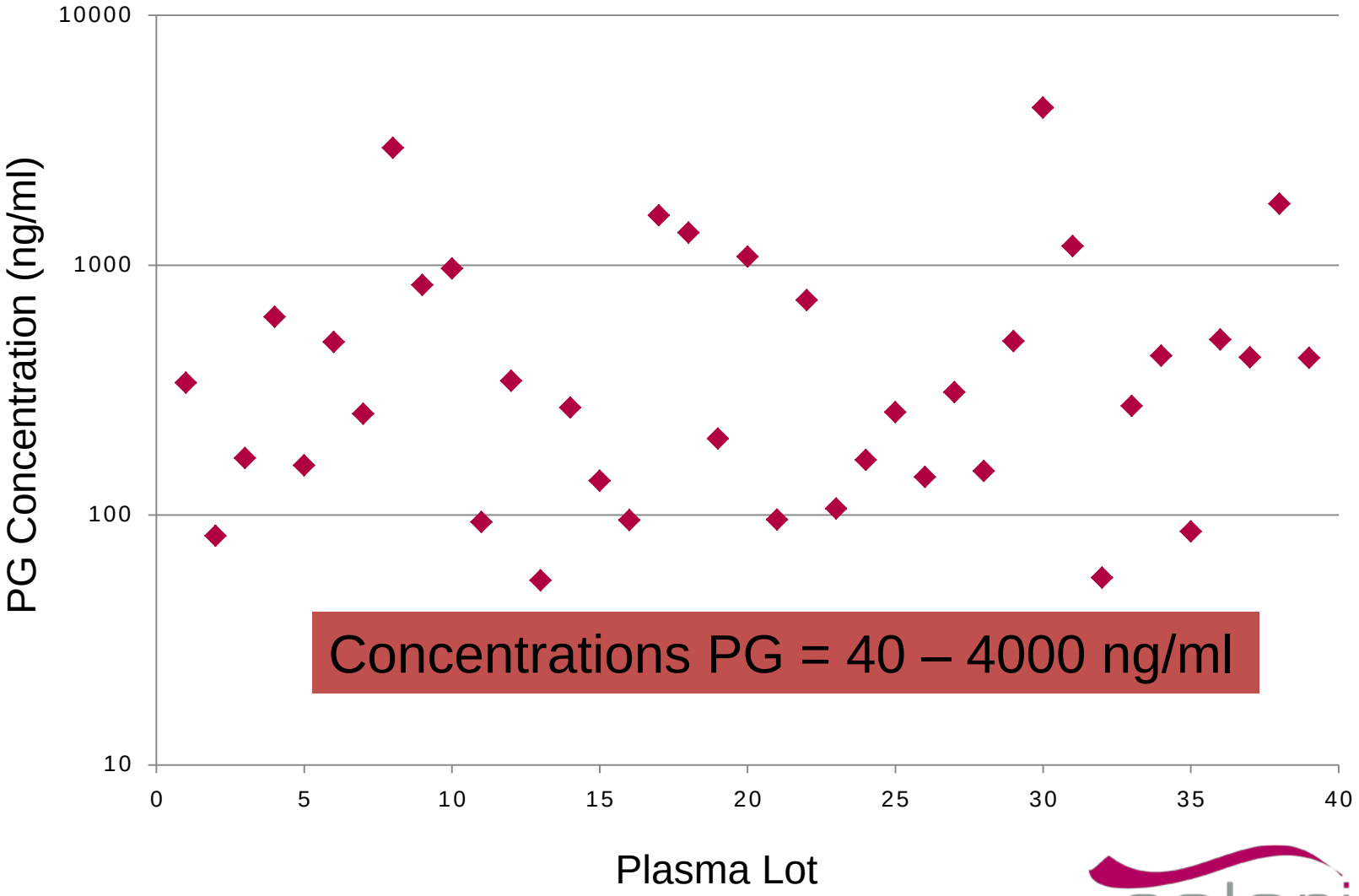
PG Calibration Curve: 100 – 10,000 ng/mL

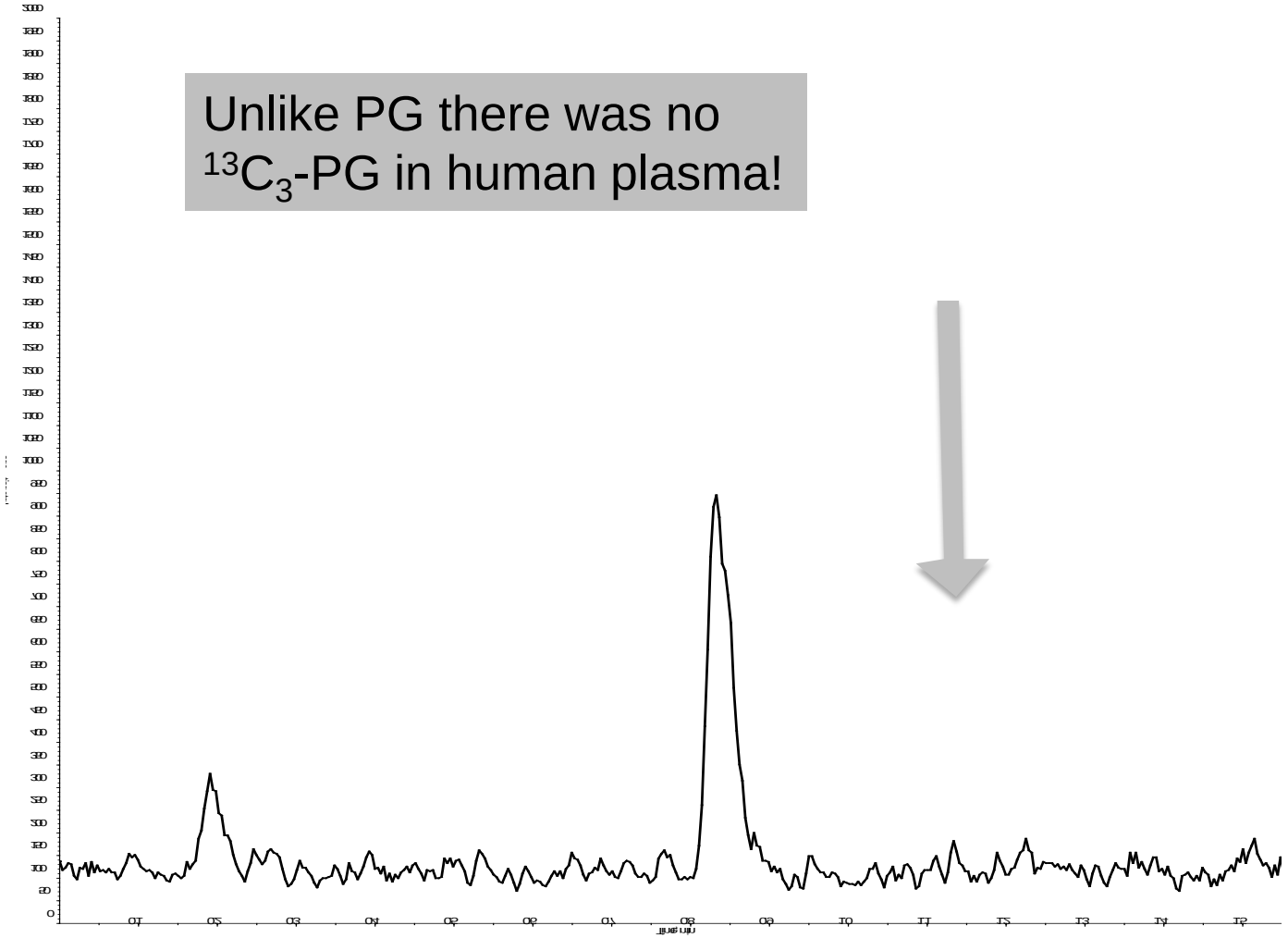


PG Concentrations in Human Plasma n = 39 plasma lots



$^{13}C_3$-PG Human Plasma Blank

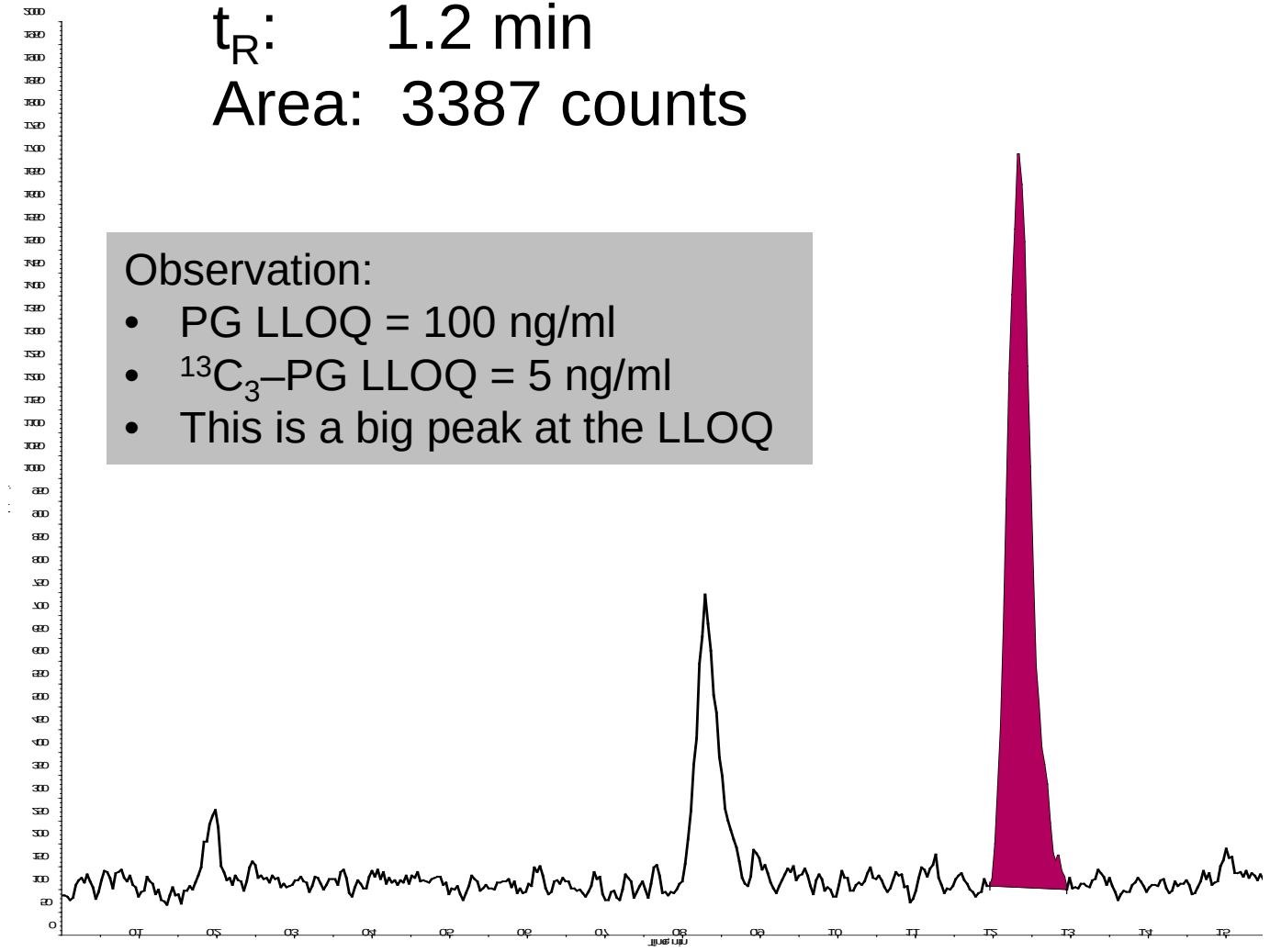
Unlike PG there was no $^{13}C_3$-PG in human plasma!



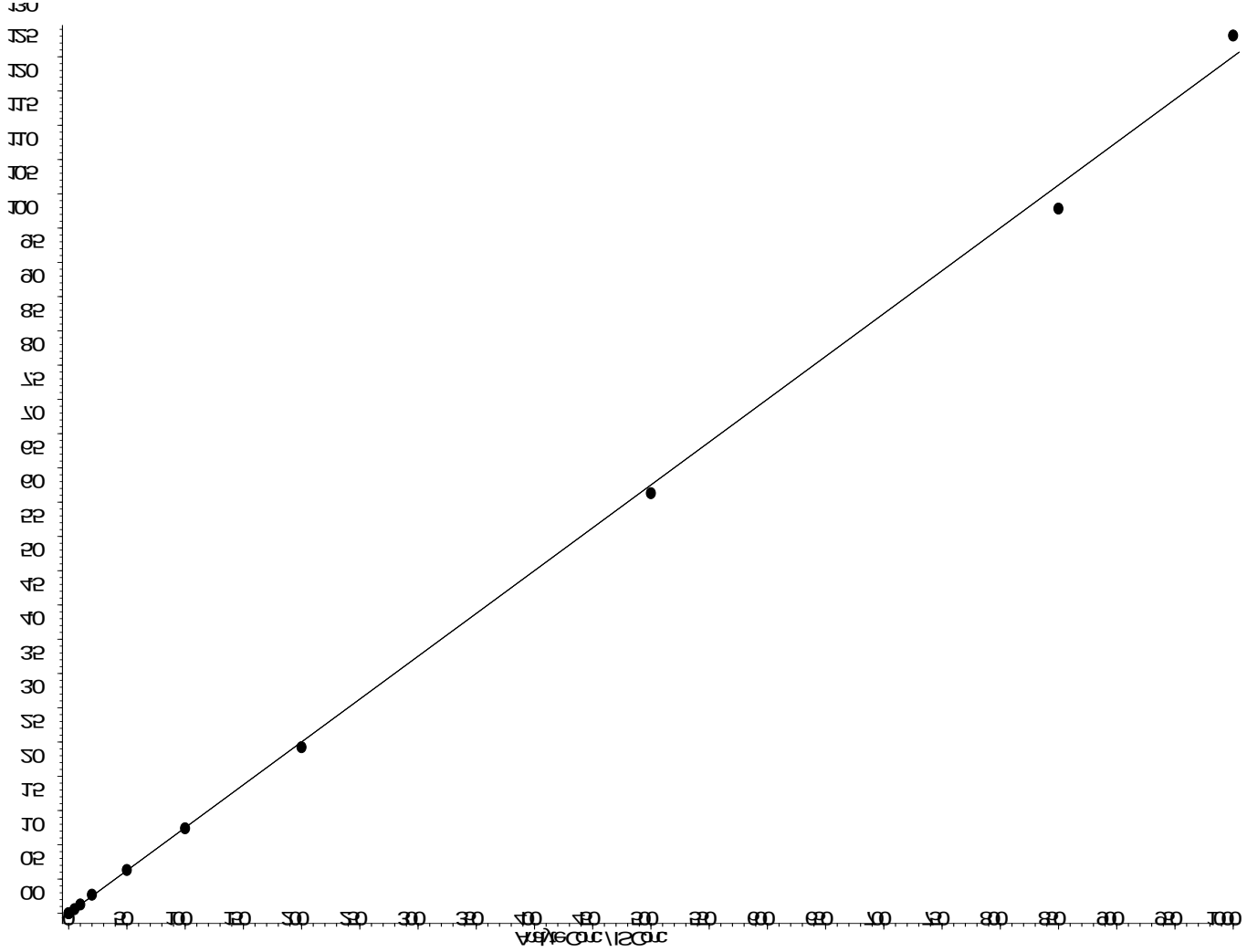
$^{13}C_3$-PG LLOQ - 5.00 ng/mL in Plasma

t_R: 1.2 min
Area: 3387 counts

Observation:
- PG LLOQ = 100 ng/ml
- $^{13}C_3$–PG LLOQ = 5 ng/ml
- This is a big peak at the LLOQ



$^{13}C_3$-PG Calibration Curve 5 – 1000 ng/mL



The power of stable isotopes in human studies APPLIED SCIENCE

- **Not radioactive** – therefore no licensing issues
- The stable isotope has the same physical/chemical properties as the non-labeled compound.
- The mass of the stable label compound is usually not endogenous
  - Example - no $^{13}C_3$-PG in blank human plasma
- Study events that could affect the non-labeled compound will not affect the stable labeled compound.

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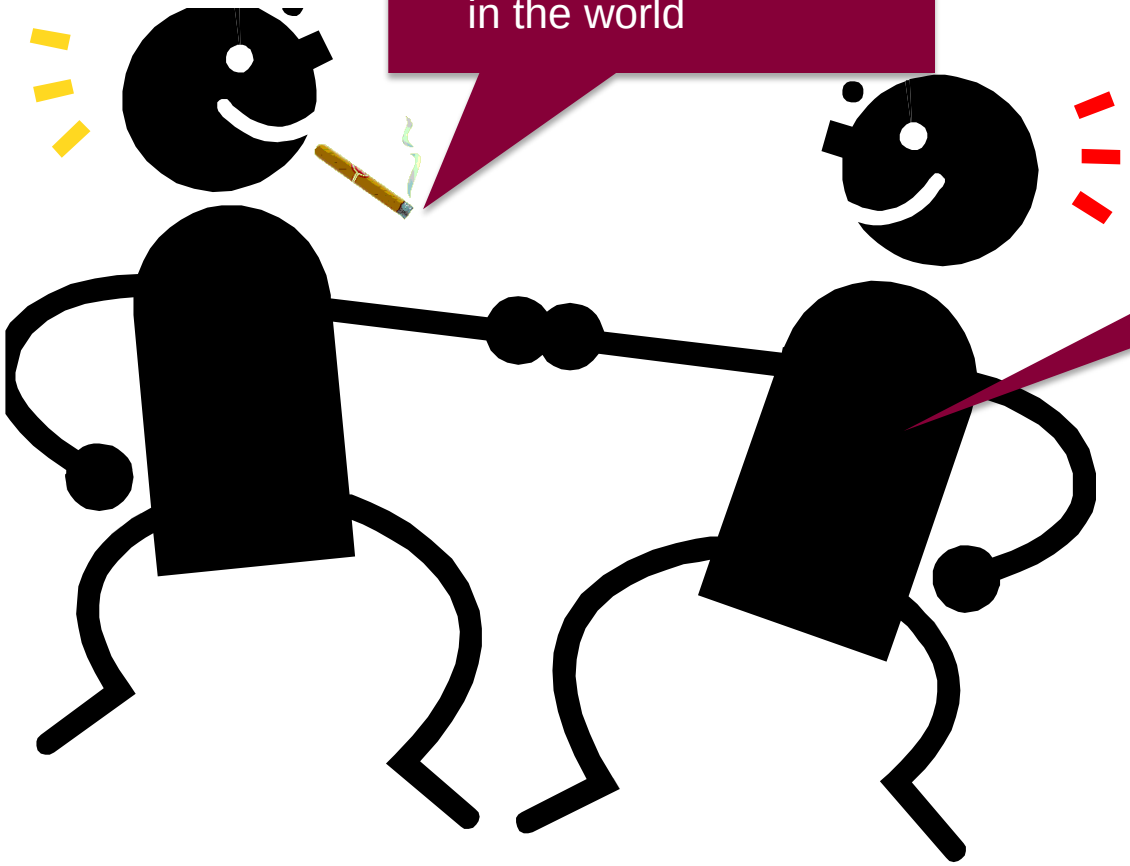
Second Hand Exposure to eCigarettes

- eCigarette filled with $^{13}C_3$-PG solvent
- Only source of $^{13}C_3$-PG in the world



If any $^{13}C_3$-PG shows up in this person's blood then it had to come from the eCigarette

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Pilot eCigarette $^{13}C_3$-PG Study: - Study Product

- KGo bottom-feeder tank with a single coil (~2.4 ohm resistance), 1100 mAh battery (~3.8 volts)
- Approximately 1 mL of solution containing 1.8% nicotine and **98.2% carbon heavy PG** loaded into the tank

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Pilot eCigarette $^{13}C_3$-PG Study: - Study Participants

All Participants	• Healthy males • 26 – 37 years of age
Vapers (n = 3)	• 6-month product use history, daily product use for 30 days prior to the test visit • No other nicotine products for the 14 days prior to the test visit • 12 hour abstention prior to test visit product administration
Non-Vapers (n = 3)	• No nicotine product use for 14 days prior to the test visit • Avoid environmental exposure to nicotine for 48 hours prior to the test visit • **USED TO TEST FOR SECOND HAND EXPOSURE**

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Pilot eCigarette $^{13}C_3$-PG Study: - Study Design

- Vaping participants self-administered one inhalation at least 3 seconds in duration every 30 seconds for a total of 30 inhalations.
- All participants were confined to a small office (21.5 m^3) for the first 2 hrs. of the study – confined space for testing second hand exposure
- Collect blood samples for 8 hours from all participants and measure:
  - nicotine
  - PG
  - $^{13}C_3$-PG

Estimated exposure by weight of vaping solution

Estimated exposure = (Pre-weight – Post-weight) X % compound

Participant	$^{13}C_3$ Propylene Glycol (98.2%)	Nicotine (1.8%)
1	239.4 mg	4.39 mg
2*	89.5 mg	1.64 mg
3	237.7 mg	4.36 mg

*Suspected device failure – the subject experienced difficulty activating the device during some of the inhalations.

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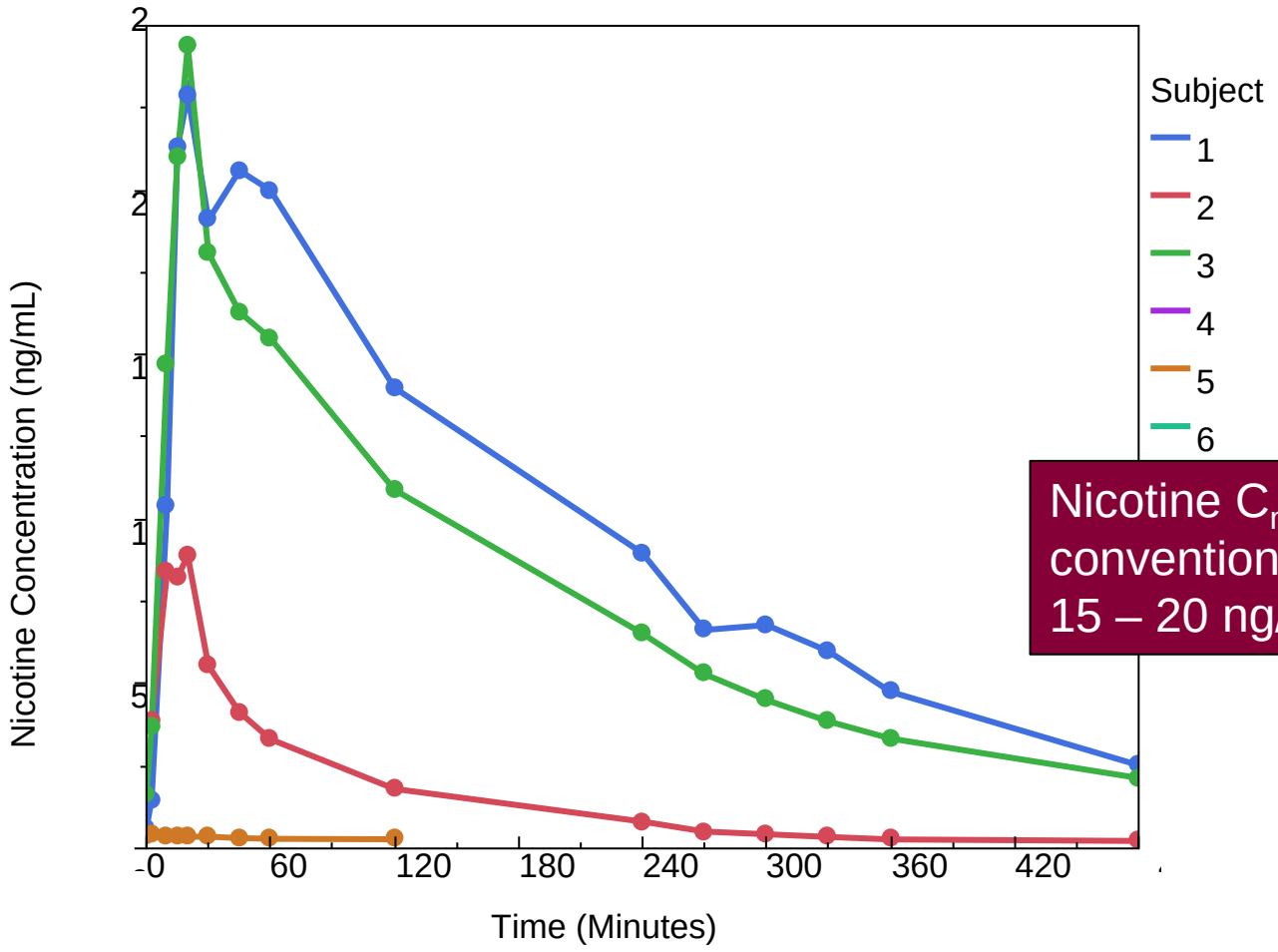
Nicotine Plasma Concentrations



Nicotine C_{max} from a single conventional cigarette = 15 – 20 ng/ml

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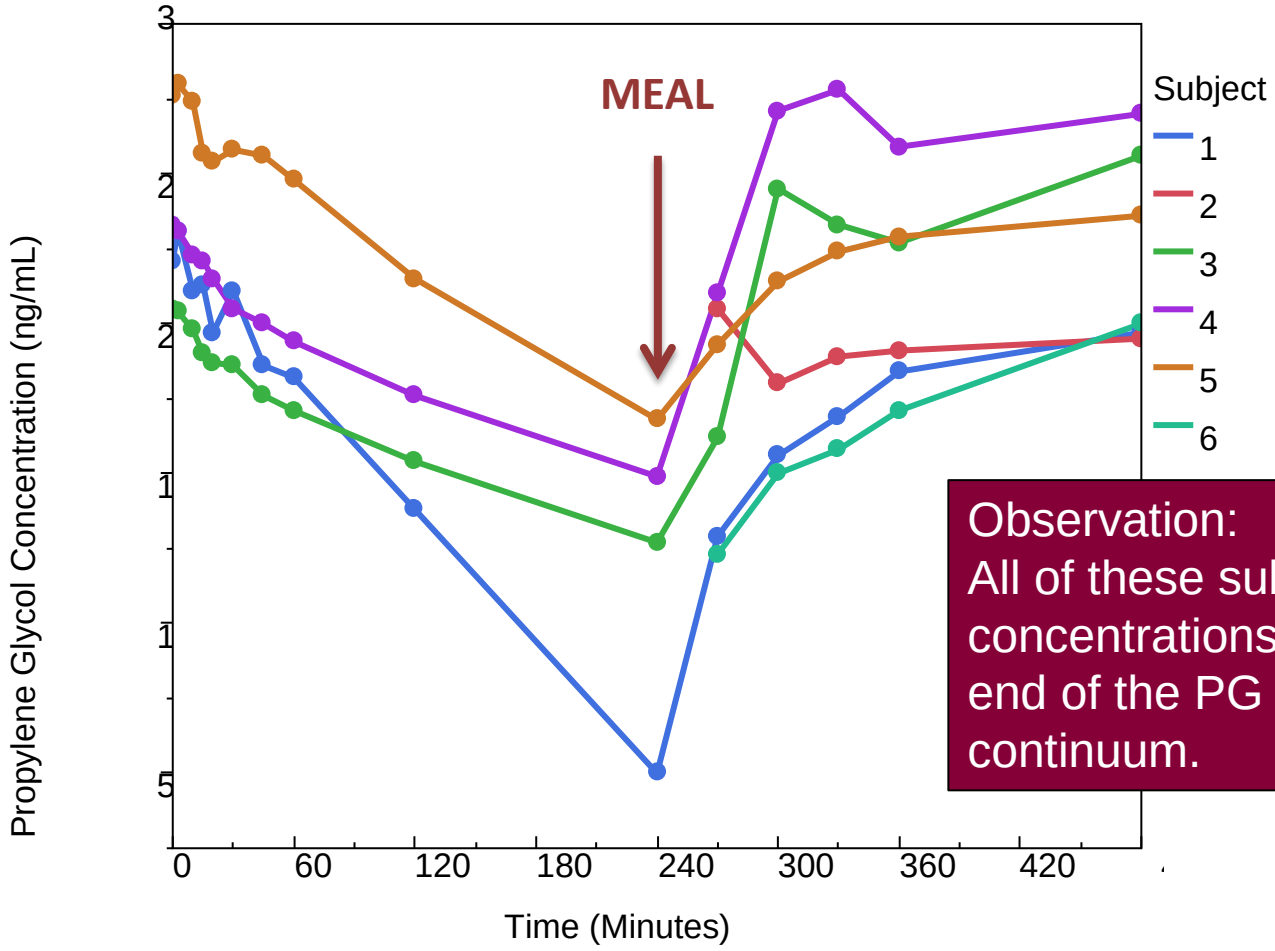
Unlabeled PG Plasma Concentrations



Observation:
All of these subjects had PG concentrations at the low end of the PG plasma continuum.

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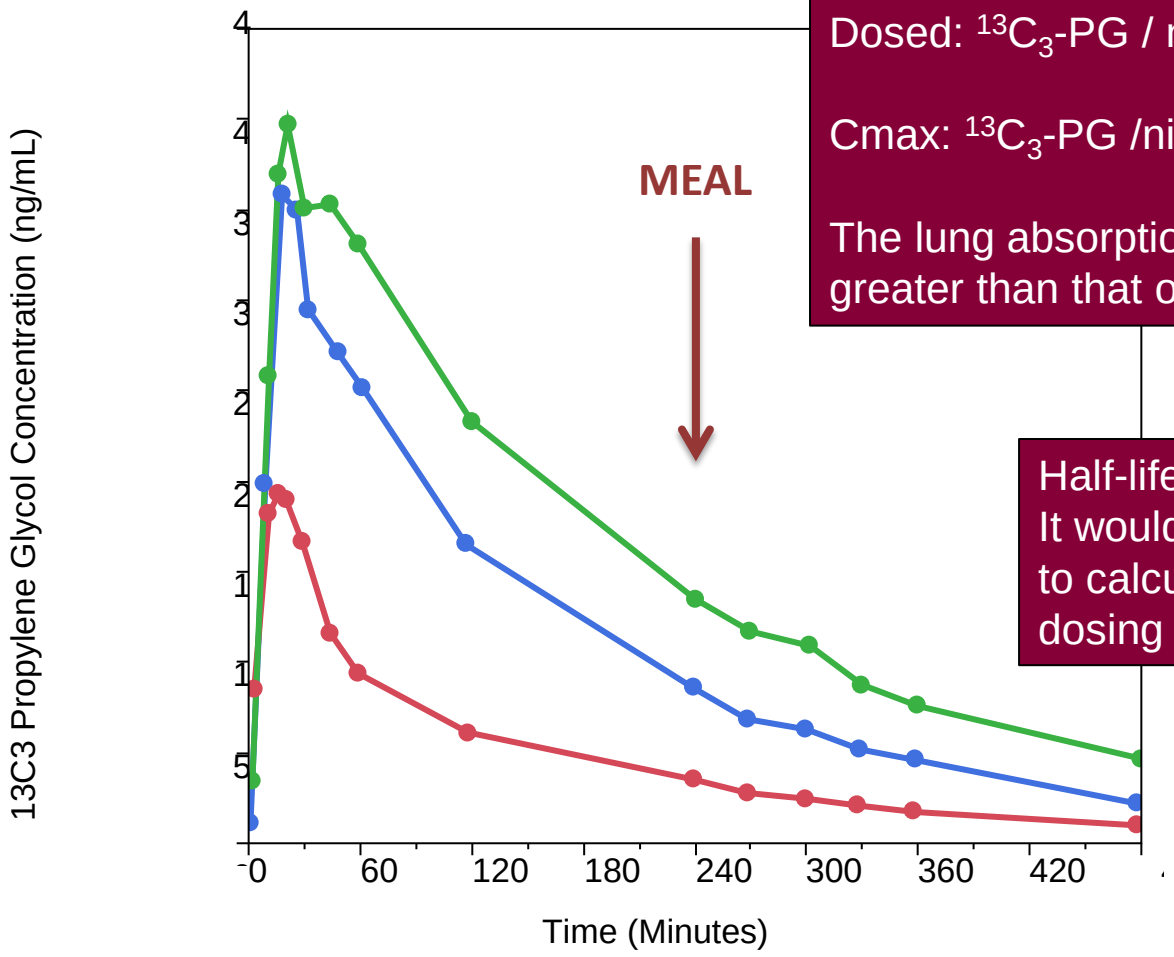
$^{13}C_3$-PG Plasma Concentrations – Vaping Participants



Observation:
Dosed: $^{13}C_3$-PG / nicotine = 50

Cmax: $^{13}C_3$-PG /nicotine > 150

The lung absorption of PG is 3X greater than that of nicotine

Half-life PG = 2 hrs.
It would be impossible to calculate this without dosing a stable isotope.

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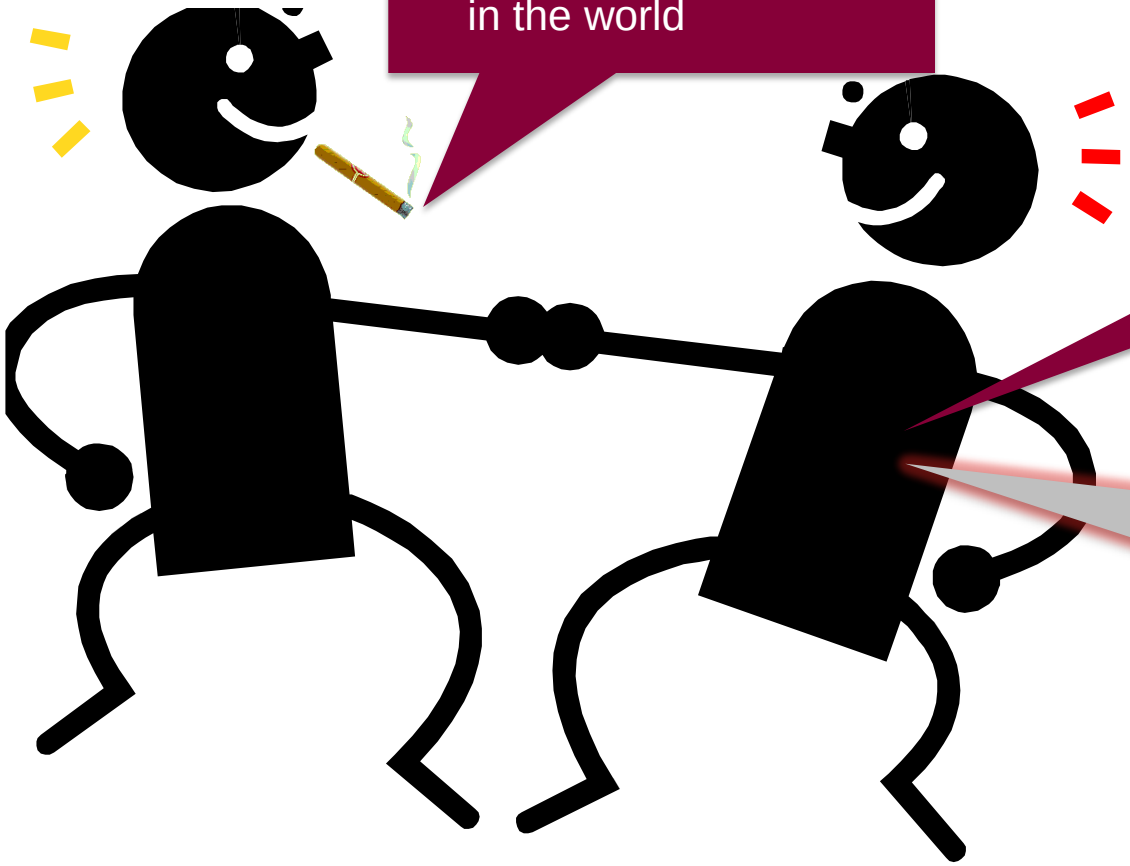
Second Hand Exposure to eCigarettes

- eCigarette filled with 98.2% $^{13}C_3$-PG
- Only source of $^{13}C_3$-PG in the world



If any $^{13}C_3$-PG shows up in this person's blood then it had to come from the eCigarette

How much $^{13}C_3$-PG showed up in this person's blood?

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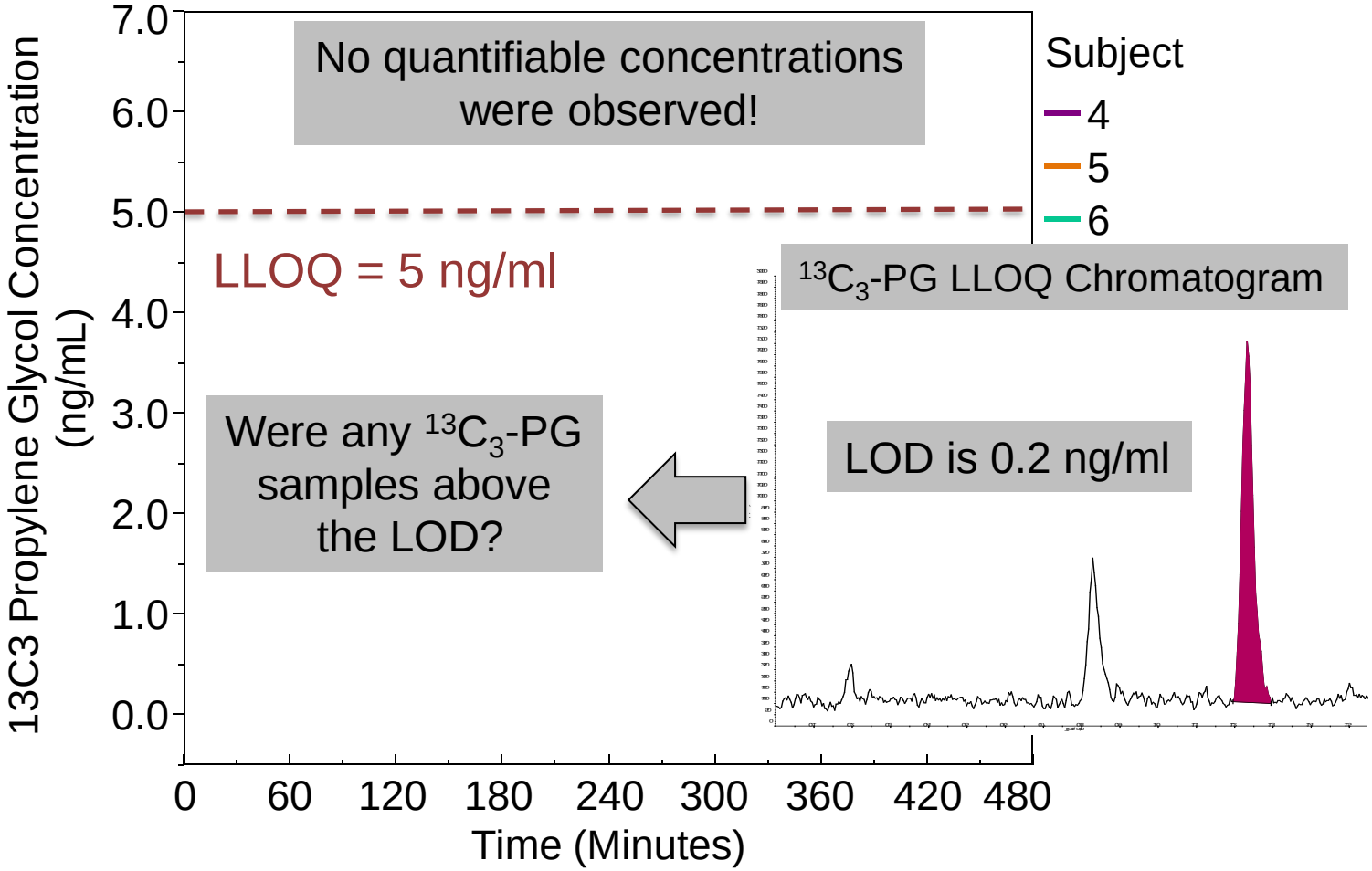
$^{13}C_3$-PG Plasma Concentrations - Non-Vaping Participants



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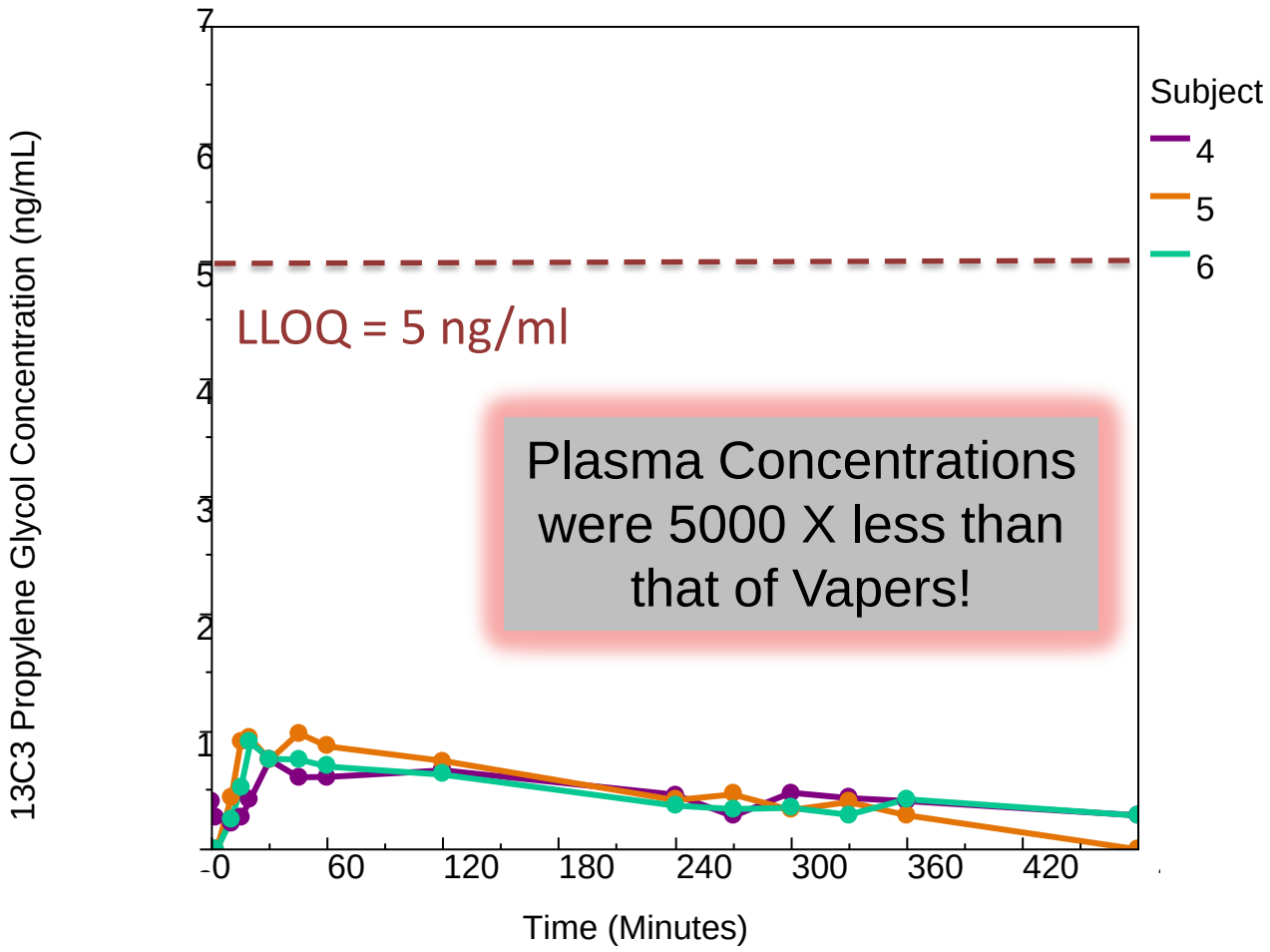
$^{13}C_3$-PG Extrapolated Plasma Concentrations below LLOQ / above LOD – Non-Vaping Participants



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Conclusions

- We have developed and validated a LC-MS/MS assay for PG and Carbon Heavy PG
- PG is ubiquitous in the environment
- PG plasma levels vary greatly between subjects
- PG plasma levels are affected by diet
- Carbon Heavy PG is a powerful tool for testing eCigarettes
  - The PG/nicotine absorption from the lungs of vapers is not linear.
- Carbon Heavy PG is a super powerful tool for testing second hand exposure to eCigarettes
- Using the Carbon Heavy PG test could have a positive affect on eCigarette legislation