



13C-Labeled Internal Standards for LC-MS/MS Analysis

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Chiron AS





Projects for developing Pharmaceutical and narcotic reference materials:

- **Industrial Ph.D (2012-2014),**
 - *The Research Council of Norway*
 - *Cooperated with NTNU*
- **ISOFOR (2012-2015),**
 - *Innovation Norway*
 - *Cooperated with NIPH*
- **Quantum SPICE (2013-2016),**
 - *EuroStars, European Funding*
 - *Cooperated with Nal Van Mitten*

THE **GOLD** STANDARD FOR FORENSIC ANALYSIS



¹³C

¹³C Labelled internal standards(IS) are superior to DEUTERATED standards in UPLC-MS/MS analysis of drugs

The same RETENTION TIMES and RESPONSE FACTORS as the natives lead to
NO BIAS WITH ION SUPPRESSION





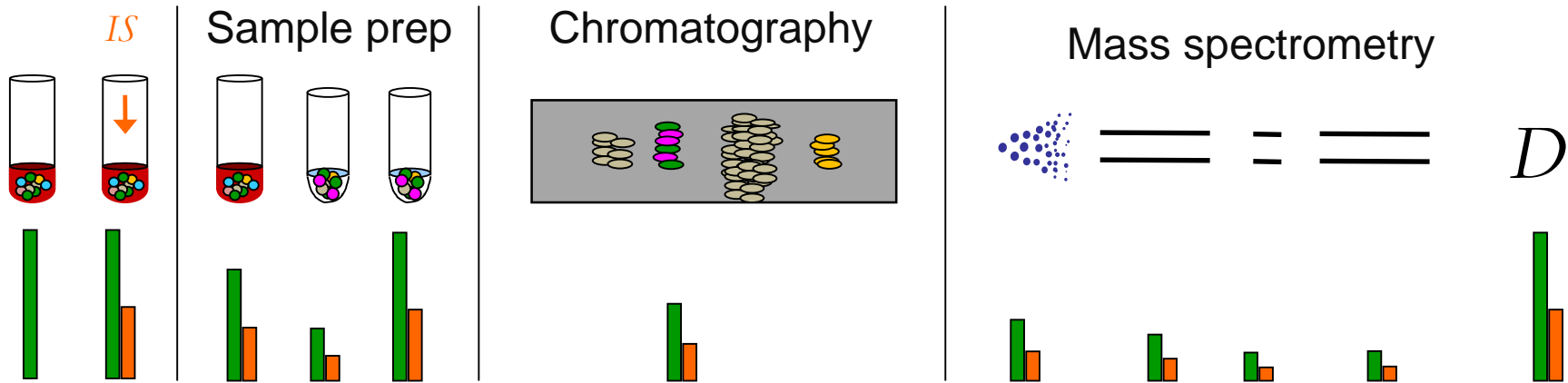
Internal Standards (ISs):

The *IS* is added prior to any manipulations in the analysis procedure

To adjust for loss and fraction, and thereby to improve qualitative and quantitative detection

Import properties for a perfect *IS*:

- Similar or identical (ideally) chemical and physical properties as the analyte
- Absent in the sample
- Detected separately from the analyte

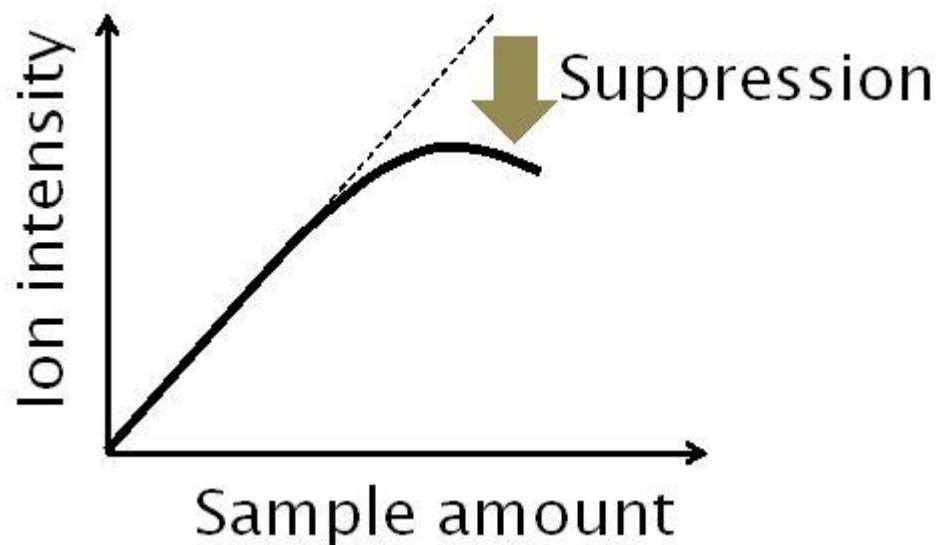


If a known quantity of IS is added to the unknown sample prior to any manipulations, the ratio of IS to analyte, remains constant, because the same fraction of each is lost in any operation.



Where can loss and fractionation occur:

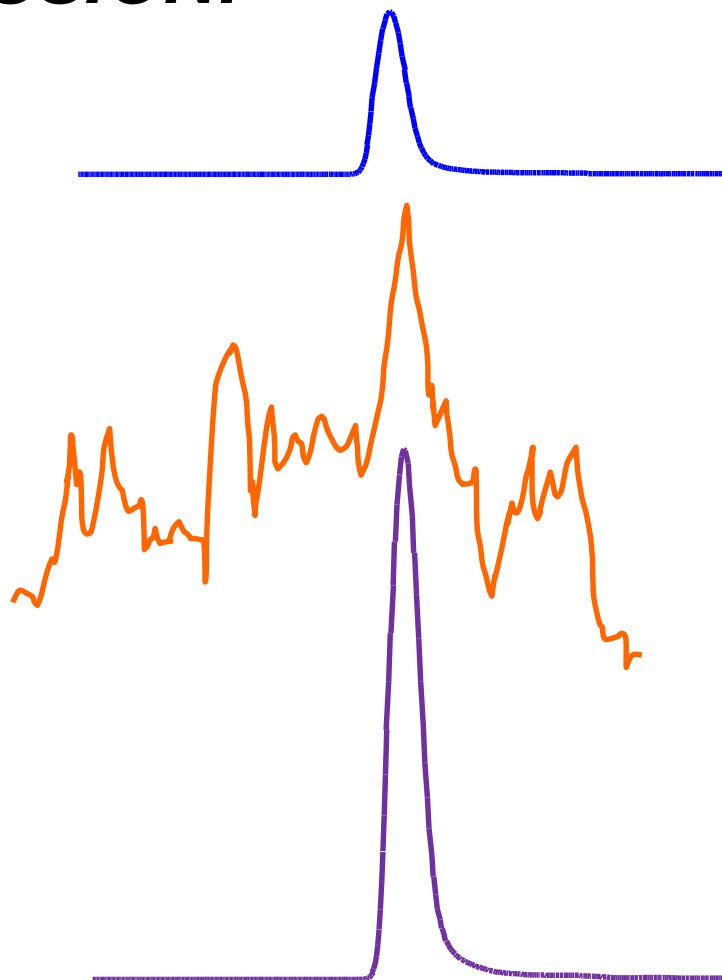
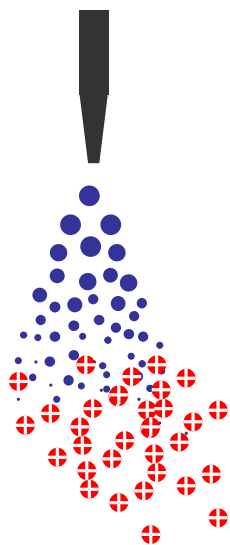
- During sample preparation and instrumental analysis
- **Ion suppression** in MS detection is a major concern





ION SUPPRESSION:

ESI



Compound of interest

Matrix
(better sample prep)

Co-eluting compounds
(better chromatography)



Stable Isotope Labeled Internal Standards - SIL-ISs

Import properties for a perfect ISs:

- Similar or identical (ideally) chemical and physical properties as the analyte
- Absent in the sample
- Detected separately from the analyte
- co-eluted chromatographically with analyte

Stable Isotope labeled ISs: ^2H , ^{13}C , ^{15}N , ^{18}O

Can all SIL-ISs be the perfect ISs?



^{13}C -labeled ISs vs. ^2H -labeled ISs

- *Results from ISOFOR, Innovation Norway Project*
- *Cooperation with Norwegian Institute of Public Health (NIPH)*

13C-labeled ISs are superior to deuterium labelled IS

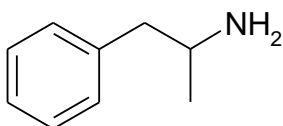
13C- labeled ISs give better:

- *Ion suppression correction*
- *Accuracy*
- *Precision*

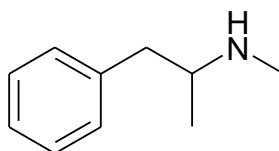




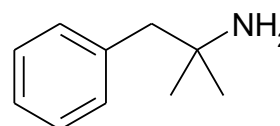
Amphetamines



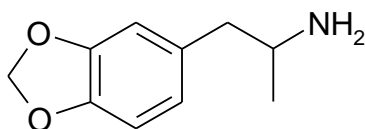
Amphetamine



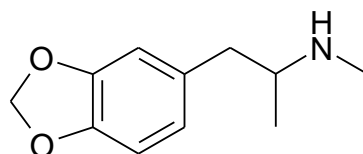
Methamphetamine



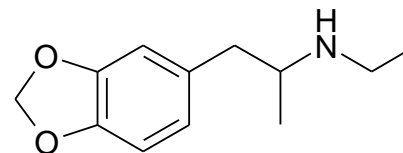
Phentermine



MDA



MDMA



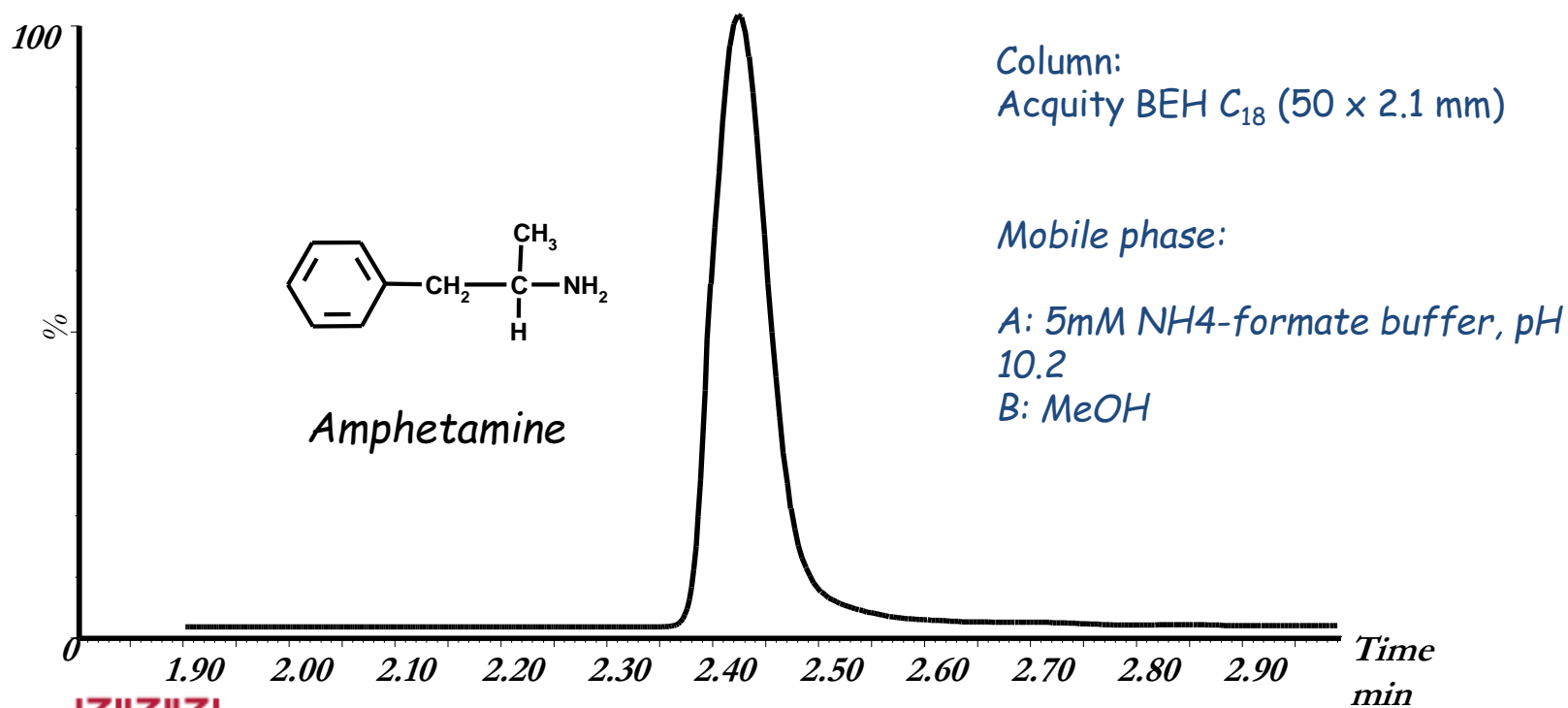
MDEA

Chiron produces native, ^2H and ^{13}C labeled standards



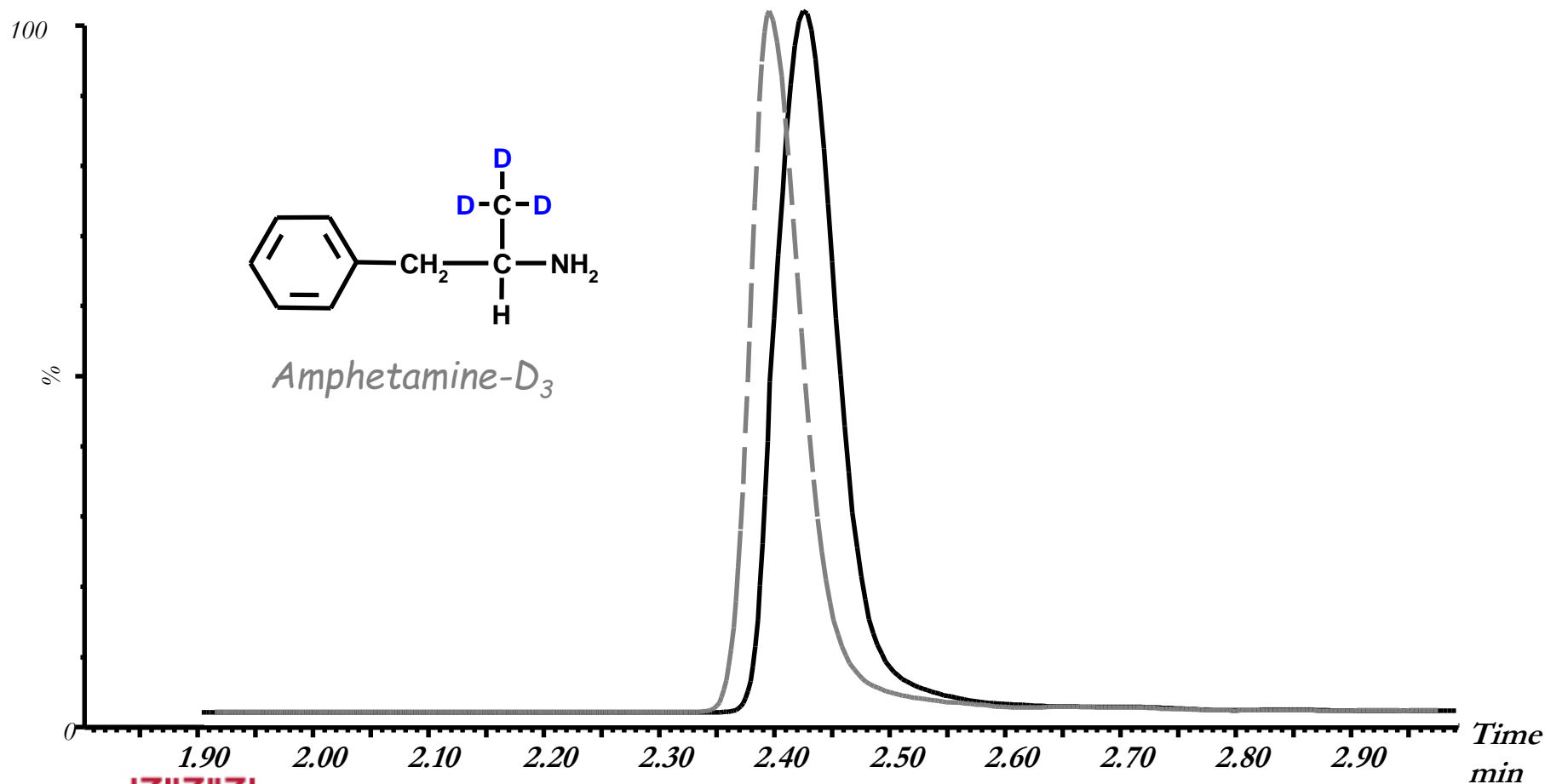
^{13}C -labeled IS vs. ^2H -labelled IS:

UPLC-MS/MS (Thomas Berg and Dag H. Strand, NIPH)



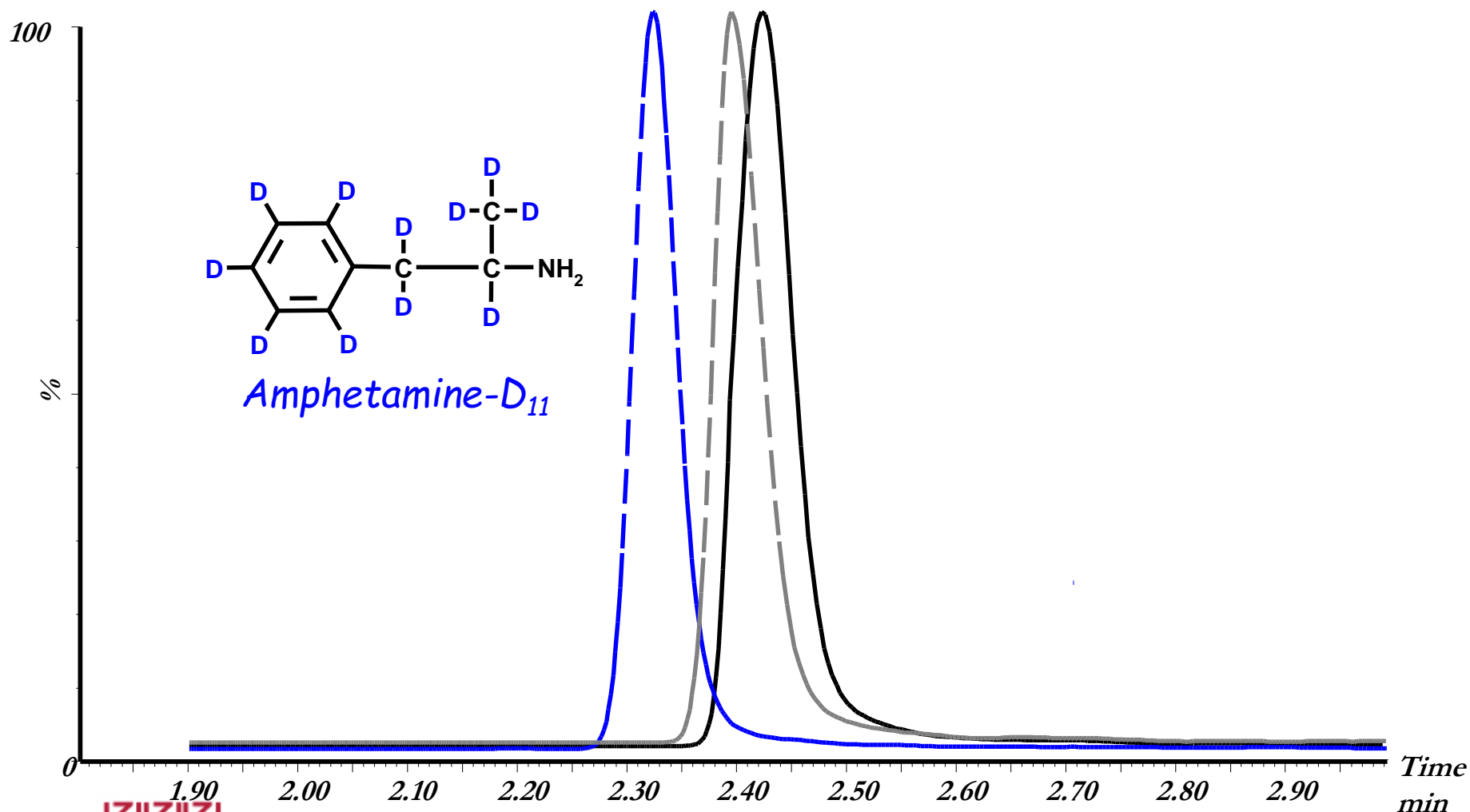


UPLC-MS/MS (Thomas Berg and Dag H. Strand, NIPH)



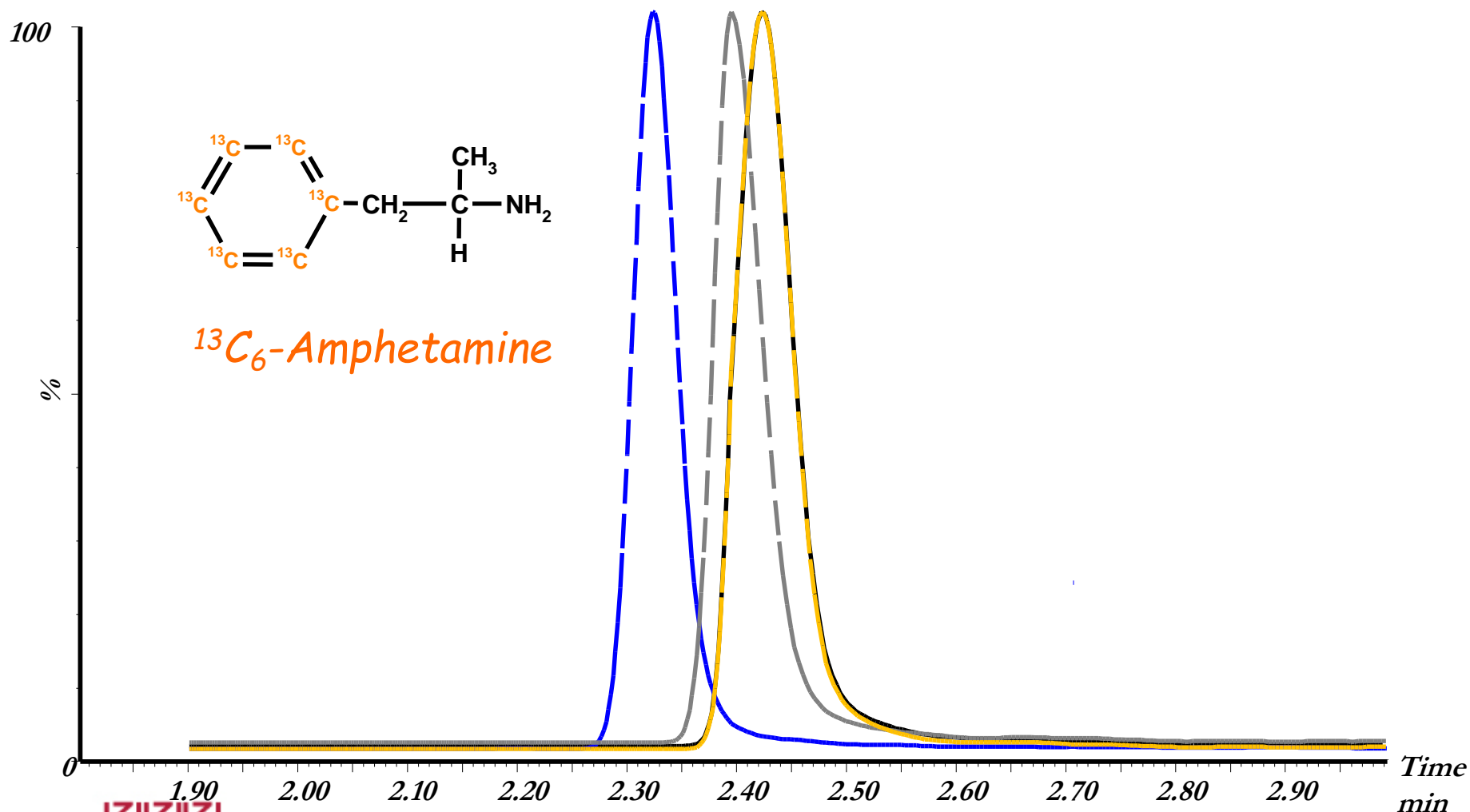


UPLC-MS/MS (Thomas Berg and Dag H. Strand, NIPH)





UPLC-MS/MS (Thomas Berg and Dag H. Strand, NIPH)



Amphetamine concentration

12 x 3 extracted standard samples

Measured Concentration

μM

1000

800

600

400

200

0

0

200

400

600

800

1000

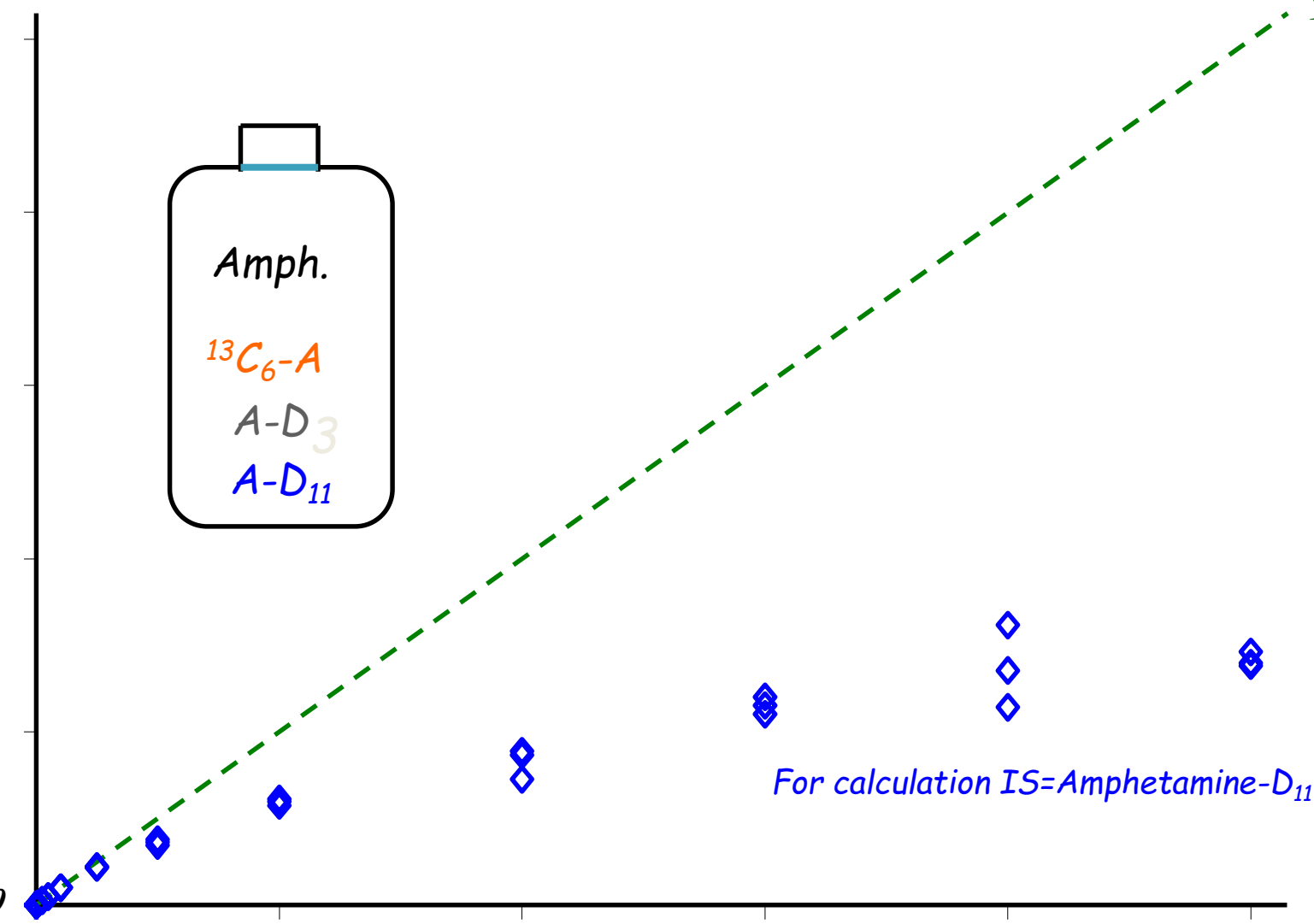
μM

Spiked concentration

$$Y = X$$

Amph.
 $^{13}\text{C}_6\text{-A}$
A-D₃
A-D₁₁

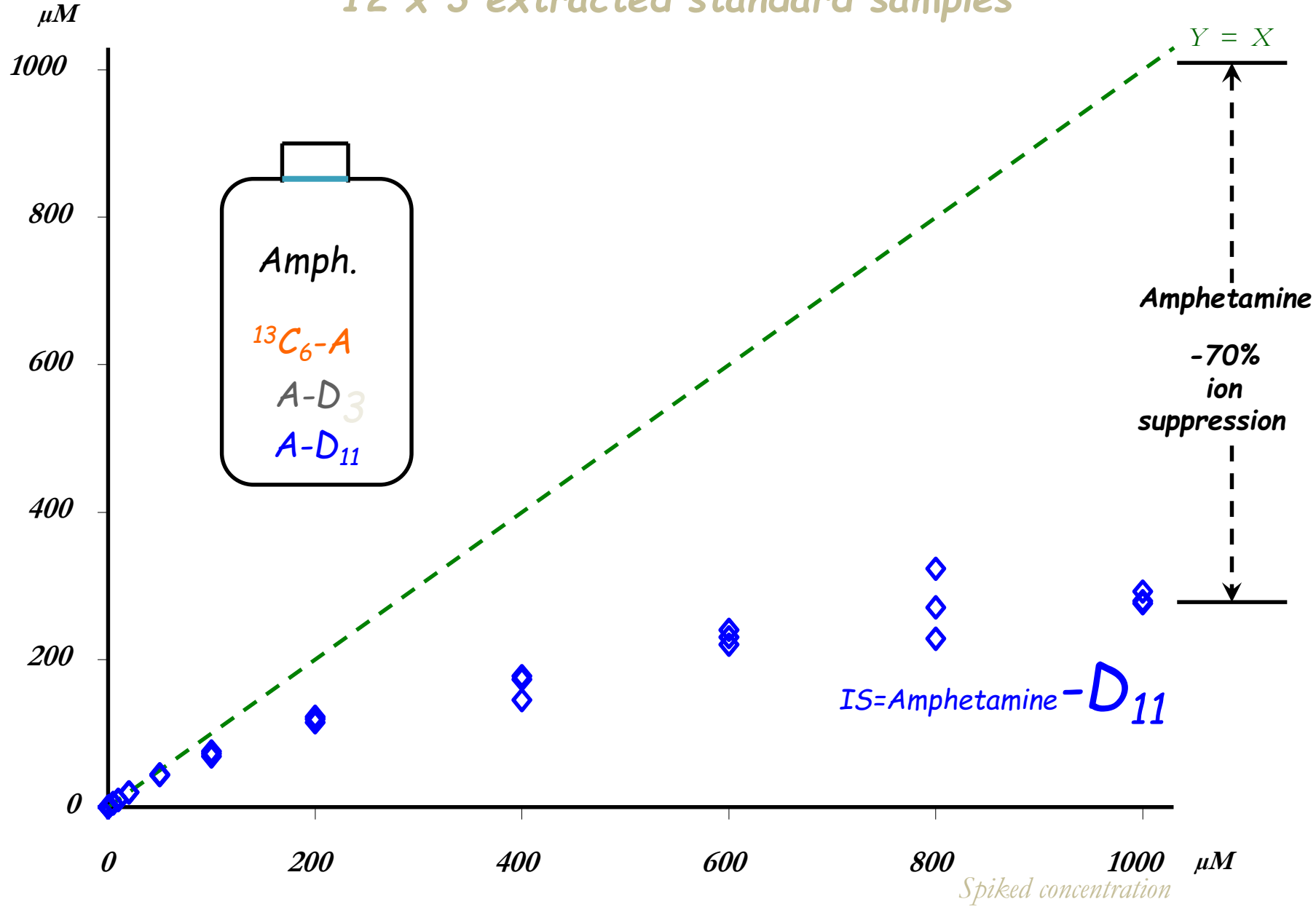
For calculation IS=Amphetamine-D₁₁



Amphetamine concentration

12 x 3 extracted standard samples

Measured Concentration

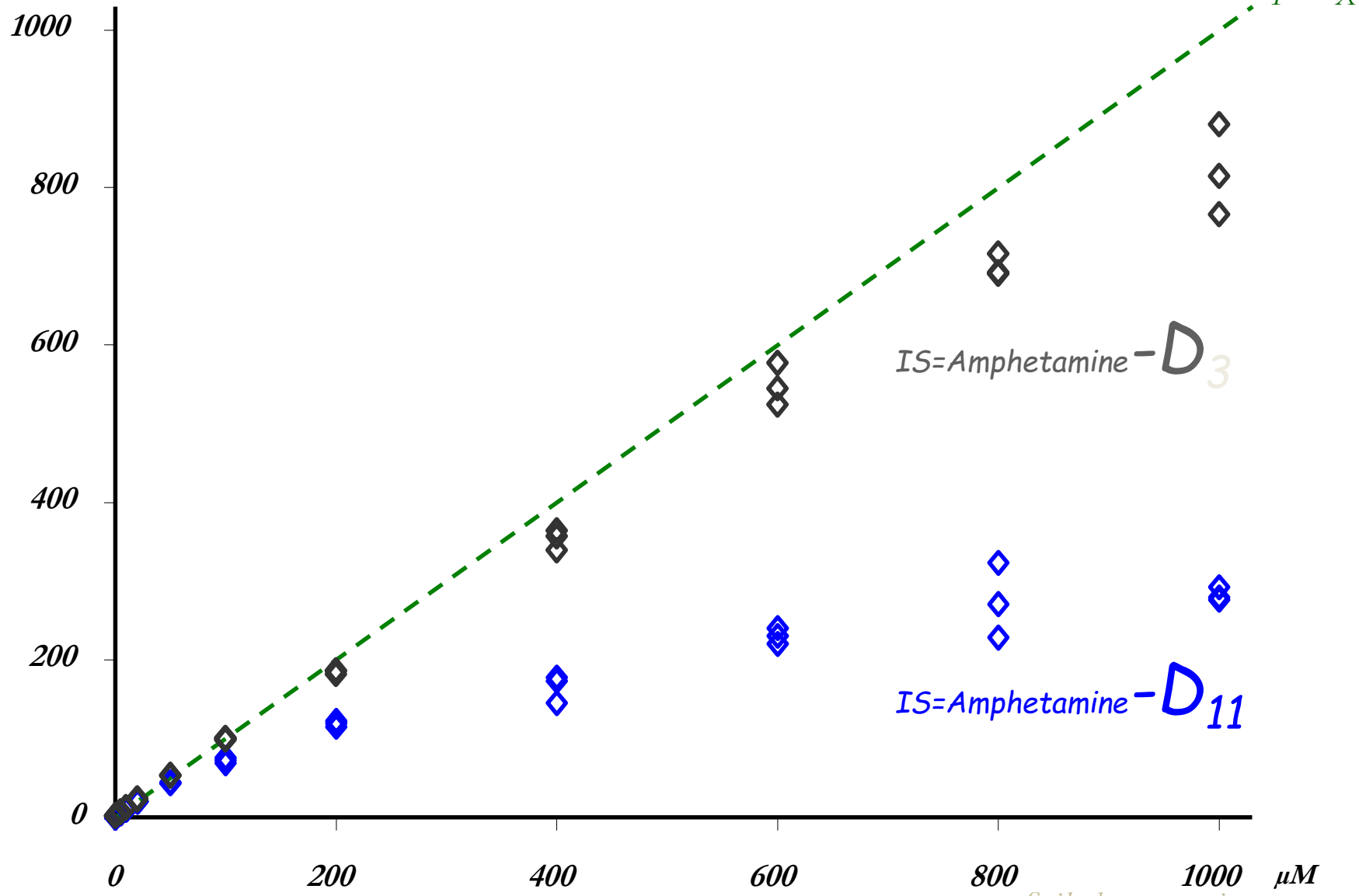


Amphetamine concentration

12 x 3 extracted standard samples

Measured Concentration

μM



$Y = X$

IS=Amphetamine-D₃

IS=Amphetamine-D₁₁

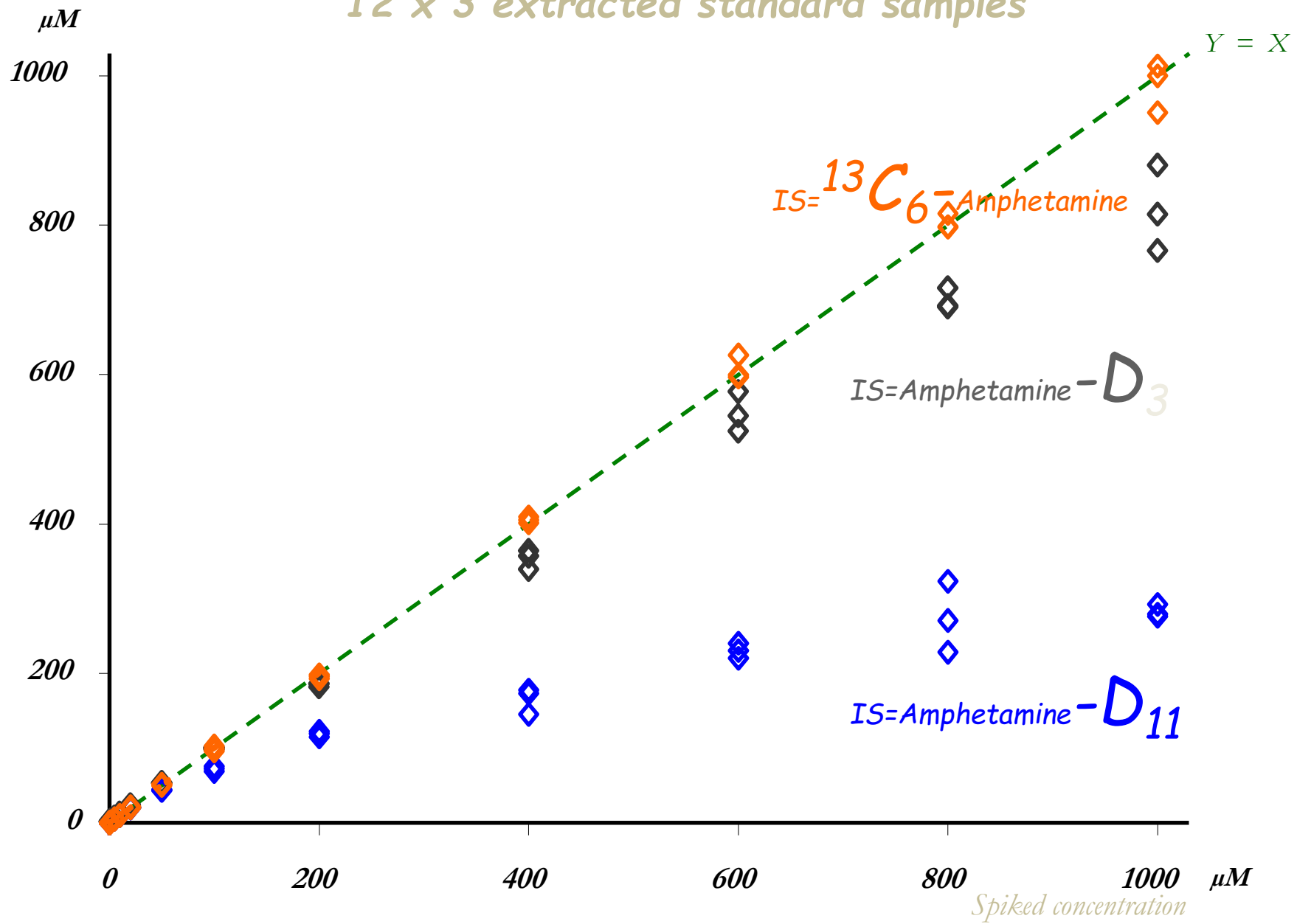
Spiked concentration

μM

Amphetamine concentration

12 x 3 extracted standard samples

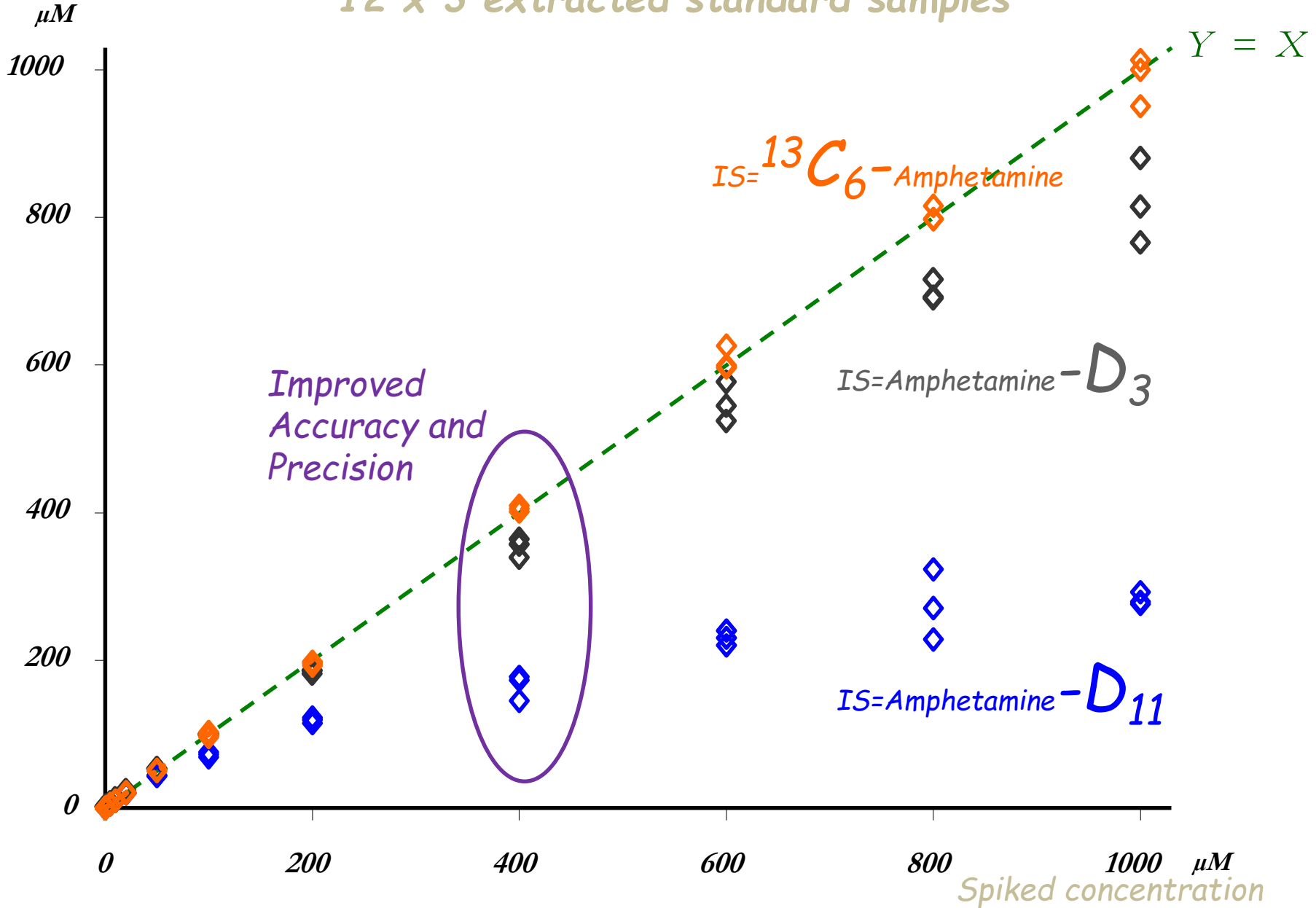
Measured Concentration



Measured
Concentration

Amphetamine concentration

12 x 3 extracted standard samples





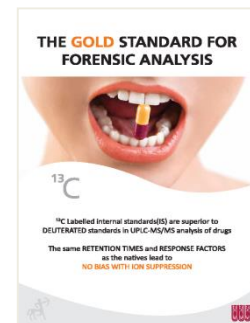
^{13}C -labeled Internal Standards (^{13}C -ISs)

- The *GOLD* Standard for LC-MS/MS

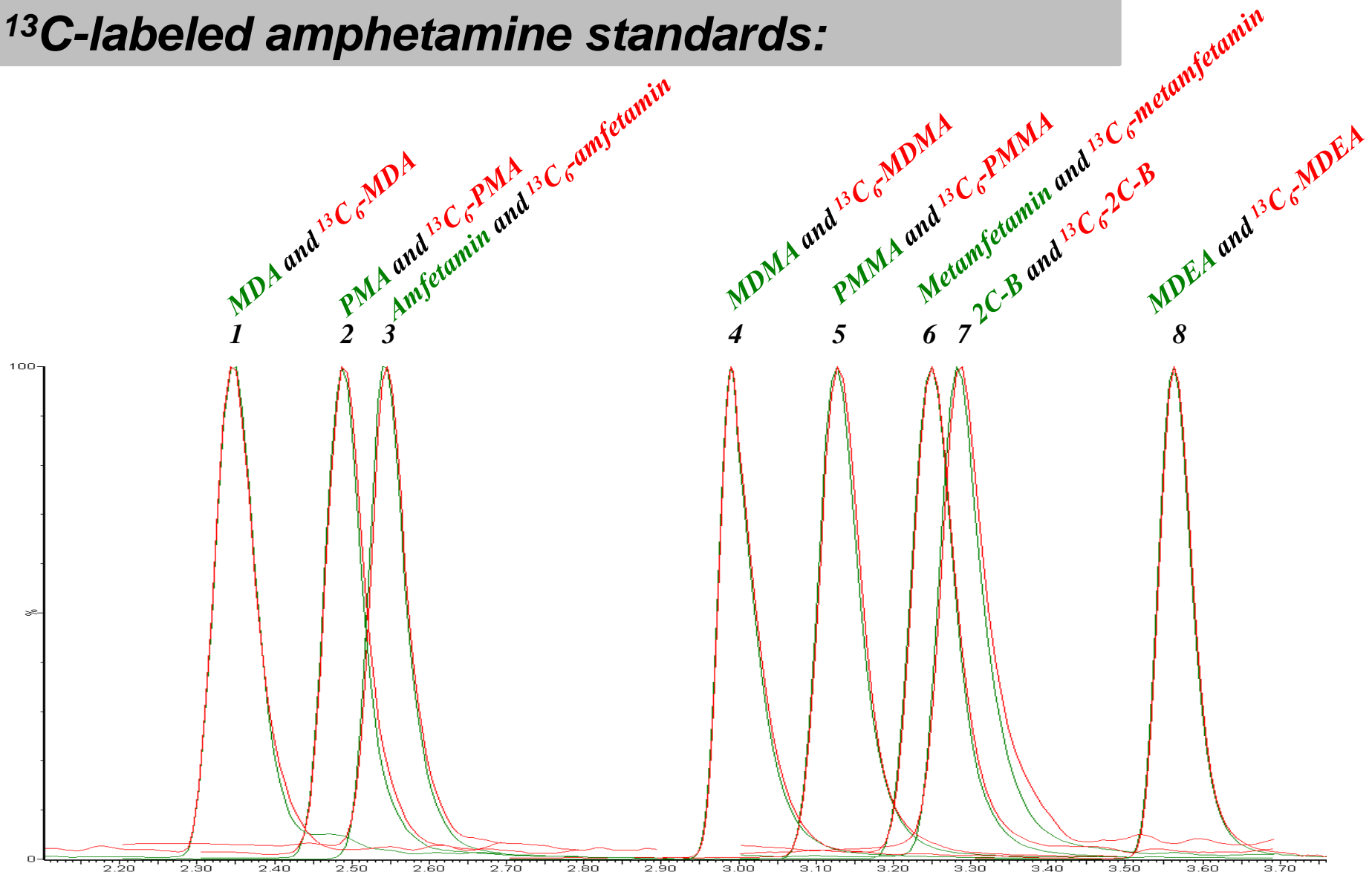
^{13}C -ISs are superior to Deuterated ISs in UPLC-MS/MS analysis of drugs

The same RETENTION TIMES and RESPONSED FACTORS as the native analytes lead to

No Bias with Ion Suppression



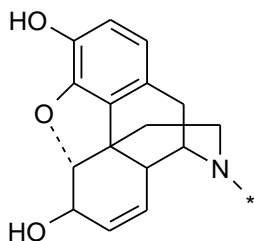
^{13}C -labeled amphetamine standards:



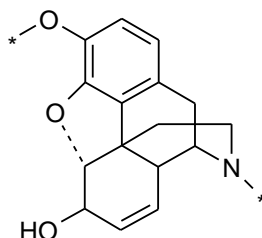
Other ^{13}C -ISs at Chiron:



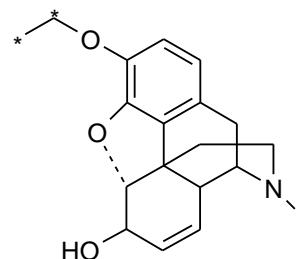
Opioids: Morphine, codeine, heroin and metabolites



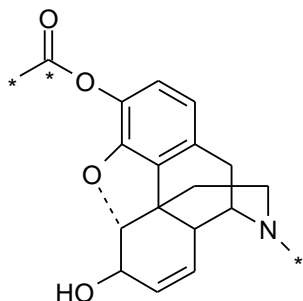
Morphine- $^{13}\text{C}_1$



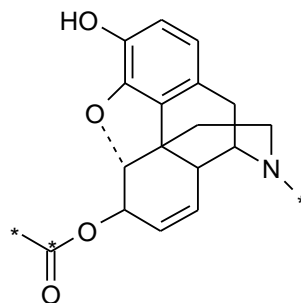
Codeine- $^{13}\text{C}_2$



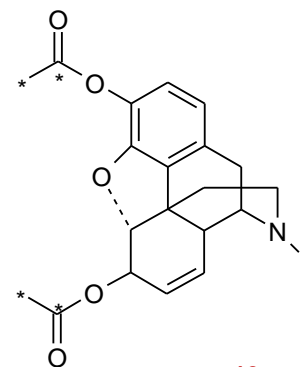
Ethylmorphine- $^{13}\text{C}_3$



3-MAM- $^{13}\text{C}_3$



6-MAM- $^{13}\text{C}_3$

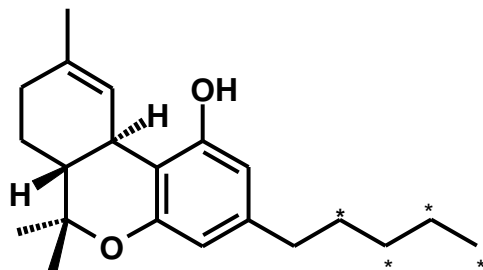


Heroin- $^{13}\text{C}_5$

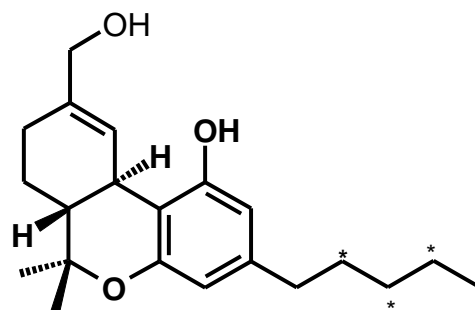


Other ^{13}C -ISs at Chiron:

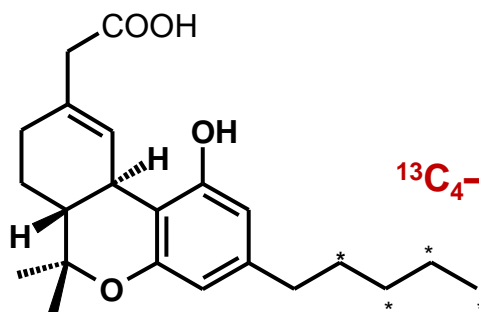
Cannabinoids: THC, THC-COOH, THC-OH



$^{13}\text{C}_4$ - Δ^9 -THC



$^{13}\text{C}_4$ -11-OH- Δ^9 -THC



$^{13}\text{C}_4$ -11-COOH- Δ^9 -THC