Practical strategies when using a “cold” isotope labeled micro tracer for aBA studies

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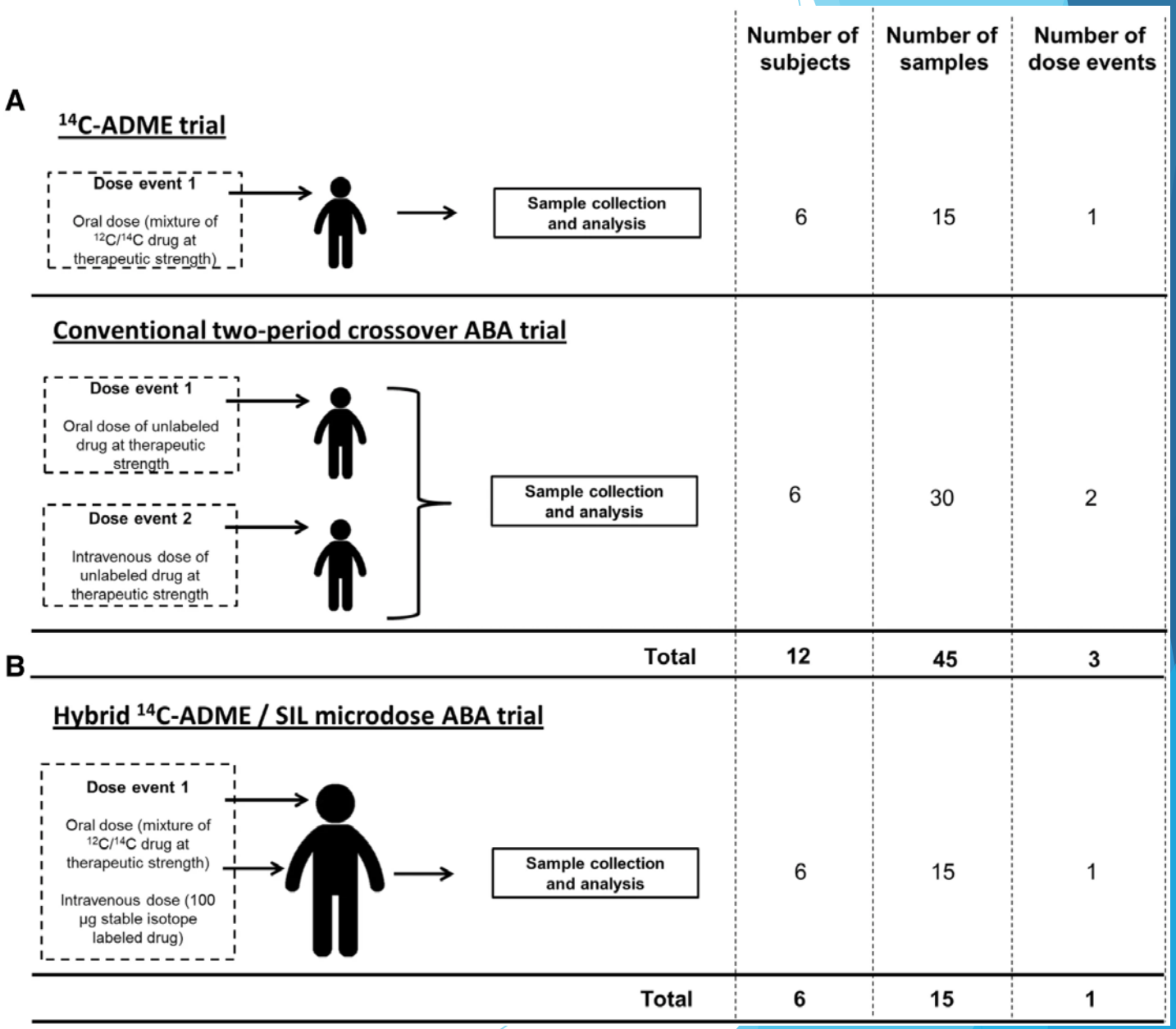
Why do we need innovative aBA study designs?

- Absolute bioavailability study measures the extent to which the active moiety of a drug is absorbed, reaches the systemic circulation.
- Absolute bioavailability (ABA) in humans is increasingly requested by the EMEA, FDA and PMDA is required for NDA submission to TGA since 2006.
- Traditional aBA study design requires IV formulation development and additional preclinical safety studies for IV route, which could cost up to $2mil and last 1yr.
- Prior knowledge of F% range is needed in order to ensure approximate same AUC for both IV and PO route and calculate bioavailability accurately

aBA study typical design

- hADME study is usually conducted together with aBA study
- Using an radiolabeled microtracer will minimize the need for preclinical dosimetry assessment
- aBA study could utilize an IV microdose of hot or cold labeled drug, minimizing safety and formulation work for IV dose
- Radiolabeled dose below 1uCi or lesser of 100ug and 1/100th of the oral dose
- Cold labeling can be used for hybrid hADME/ABA trial.

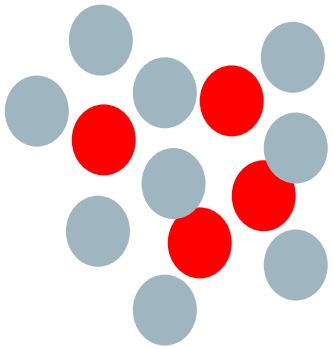


	Number of subjects	Number of samples	Number of dose events
	6	15	1
	6	30	2
Total	**12**	**45**	**3**
	6	15	1
Total	**6**	**15**	**1**

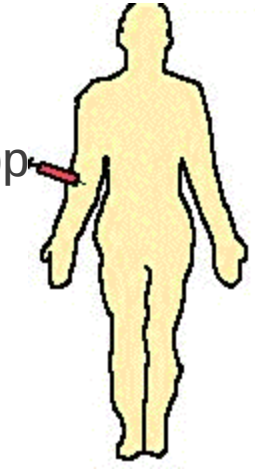
C13 labeled compound for IV dosing in aBA study

Approach 1

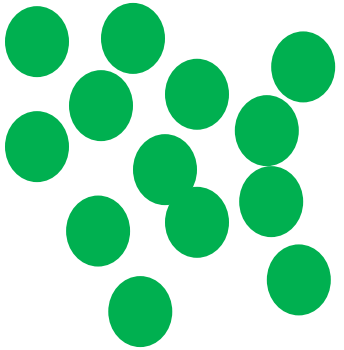
Hot API: Mixture of C14 labeled and non-labeled



App

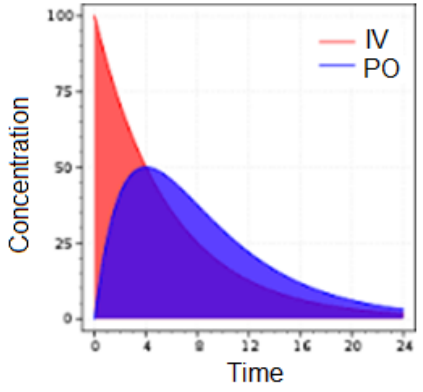


C13 labeled API: homogeneously labeled with C13



Cold API

Hot API



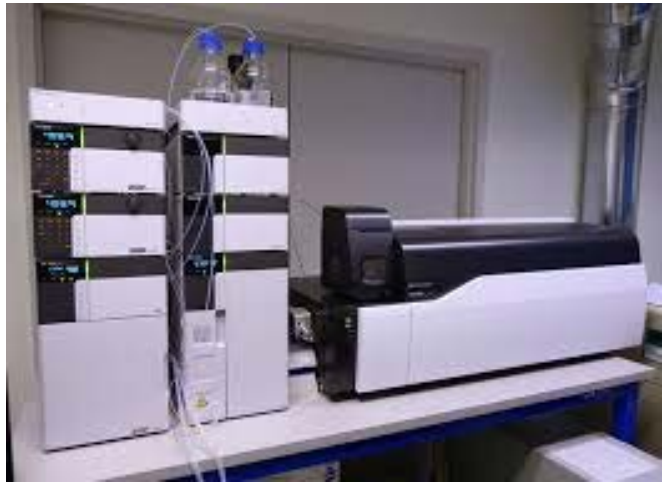
Isotope labeled API

$$F = \frac{AUC_{Oral} / Dose_{oral}}{AUC_{IV} / Dose_{IV}}$$

Considerations for selecting labeling type

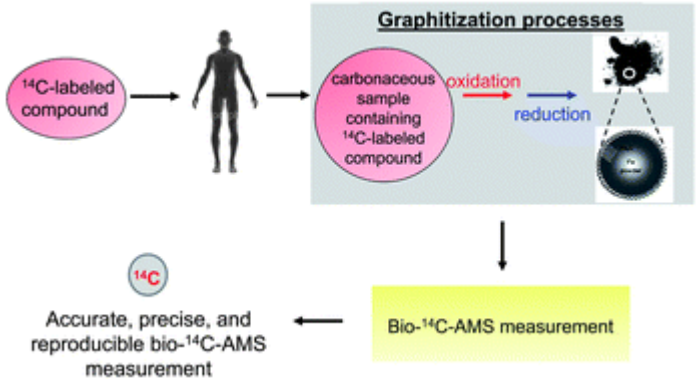
Considerations	C14	C13/N15
Cost	~$500,000	<$150,000 for GDC-0810
Dose	1μCi	100μg
Chemistry	One atom	5-10 atom
Bioanalysis	AMS	LC-MS
Example	Many drugs since 1975	Daclatasvir, tofogliflozin, evacetrapib, ibrutinib

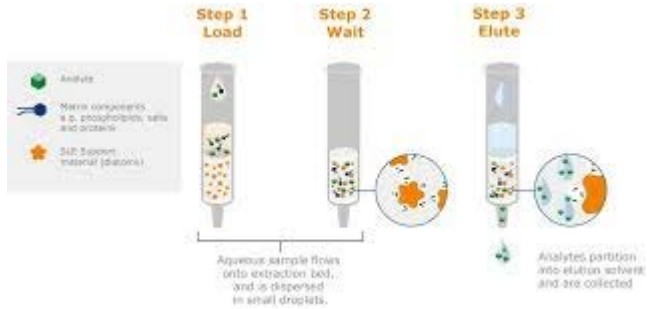
AMS process(C14) vs LC-MS(cold label)



- Sophisicated instrumentation;
- Only a few vendors worldwide

- Widely available instrumentation;
- Many CRO has the capability



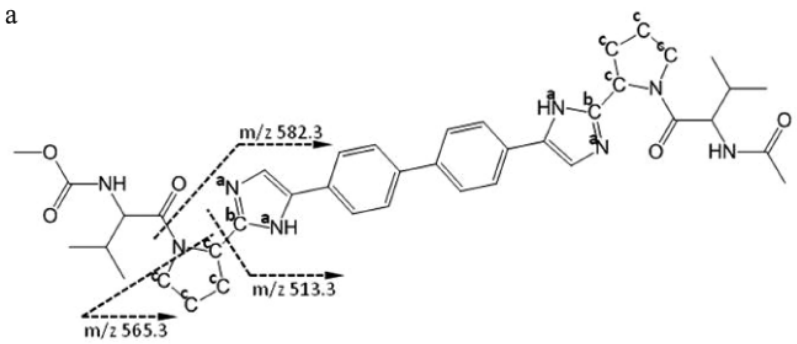


Lengthy manual preparation

Highly automatable process

First case of cold labeled aBA study: Daclatasvir

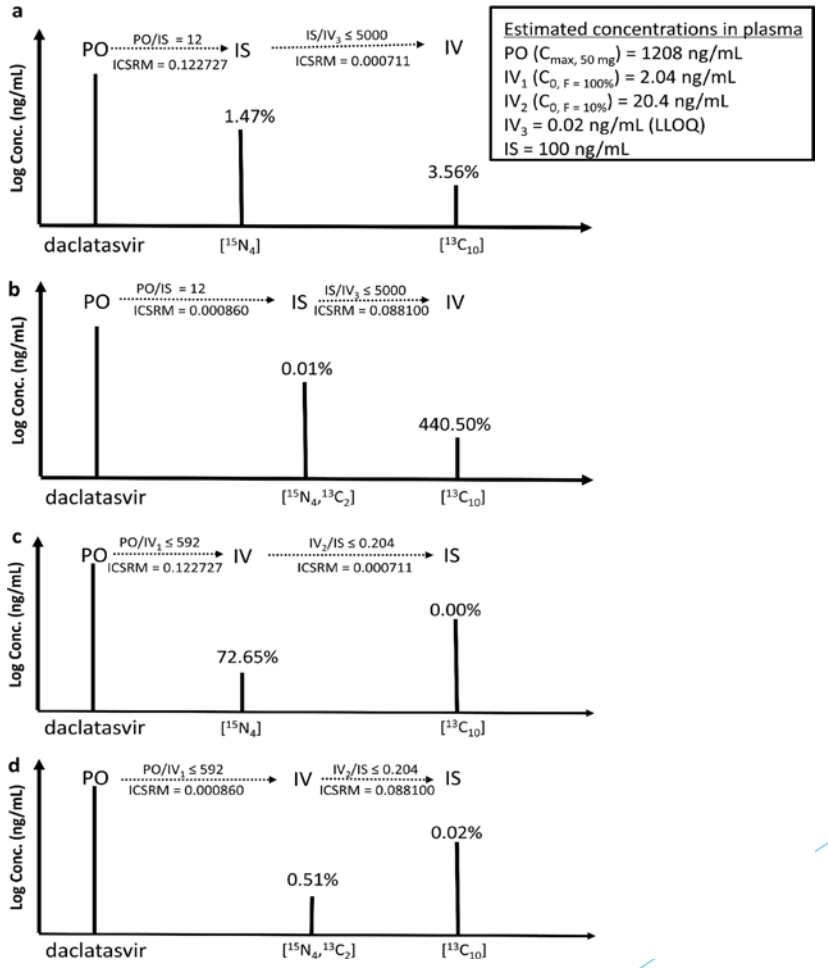
- NS5a inhibitor for HCV, 60mg QD



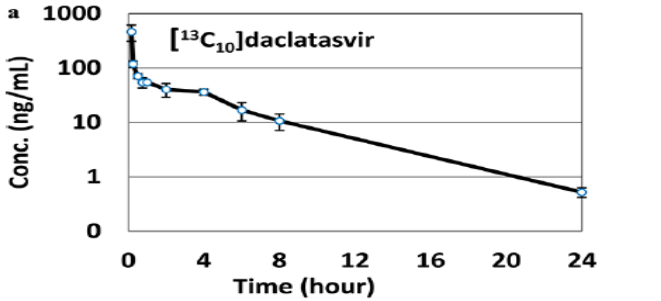
[$^{15}N_4$]daclatasvir (a = ^{15}N)

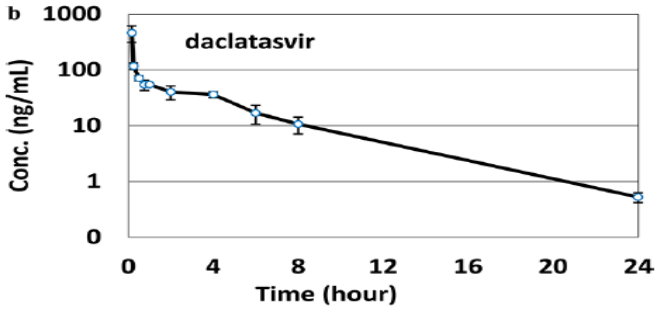
[$^{15}N_4$,$^{13}C_2$]daclatasvir (a = ^{15}N, b = ^{13}C)

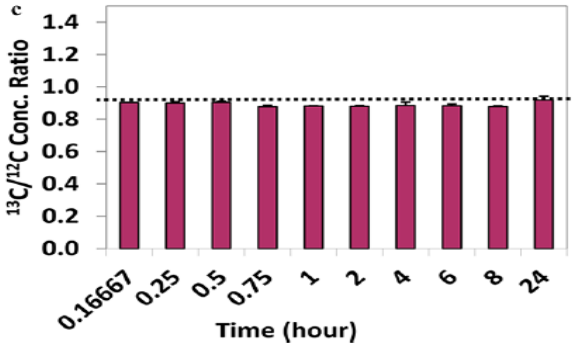
[$^{13}C_{10}$]daclatasvir (b = ^{13}C, c = ^{13}C).

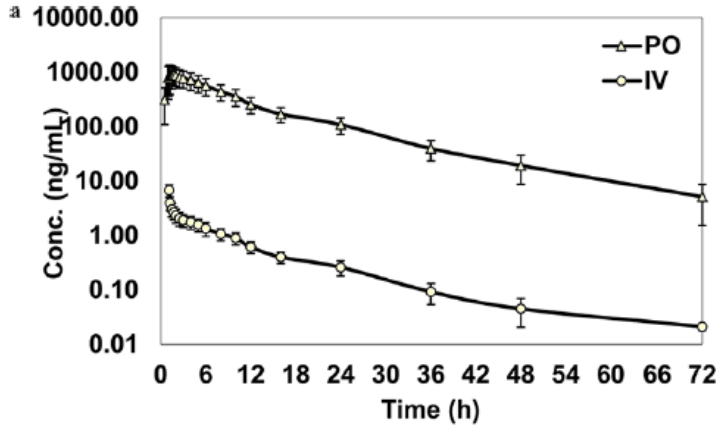


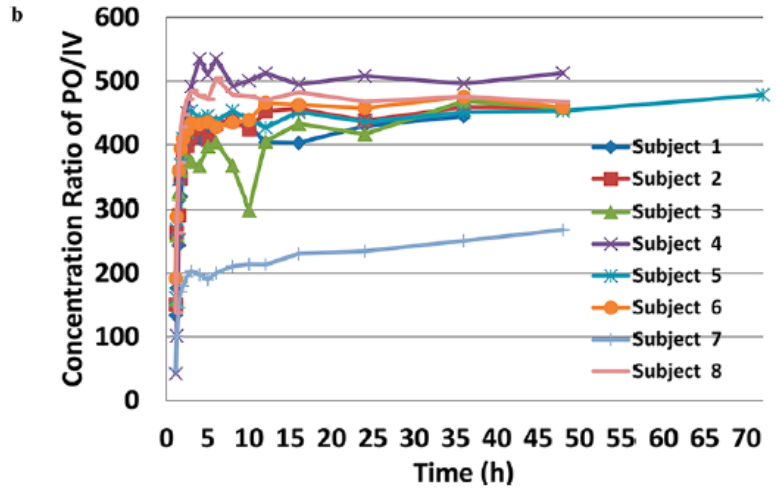
First case of cold labeled aBA study: Daclatasvir





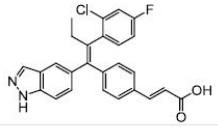






- Monkey study(on the left) to demonstrate lack of isotope effect in vivo
- Clinical study(on the right) for aBA evaluation

GDC-0810: clinical development plan



SERD1/SERM

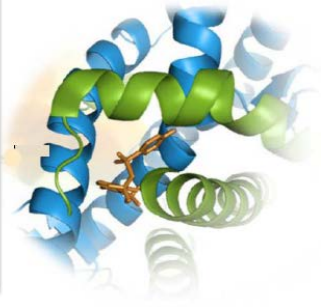
ARN-810

ERα Antagonist

Degrades ER

Target: ER+ Breast Cancer

Phase I

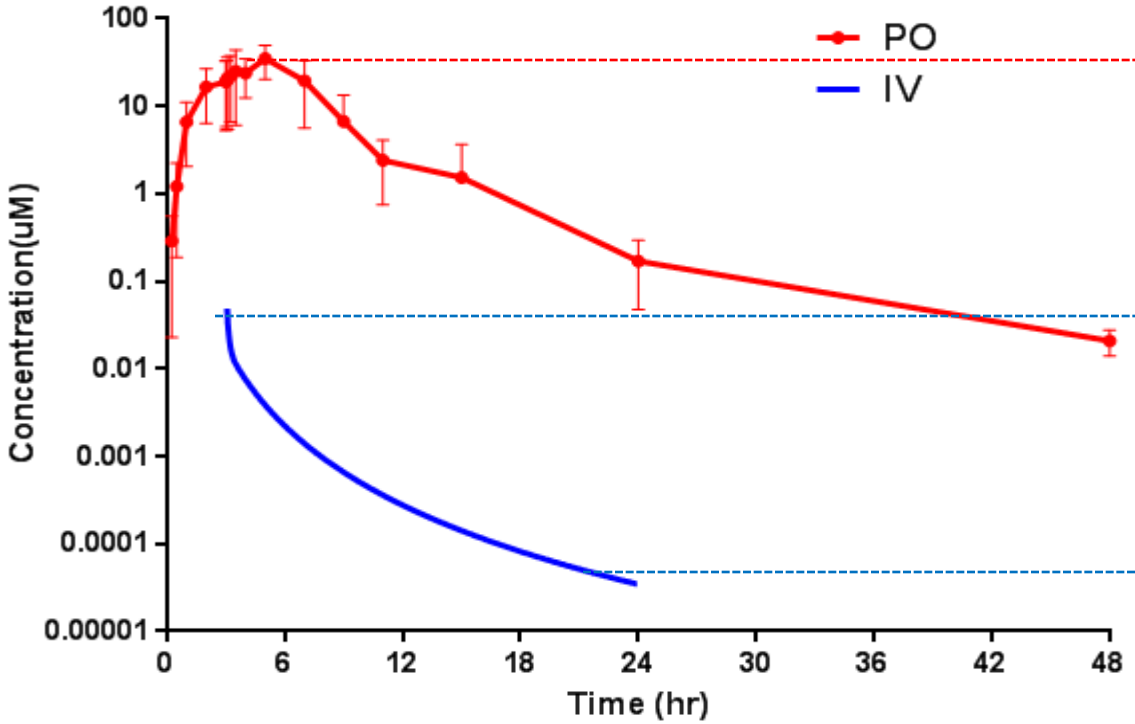


- Target patient: post menopausal women, ER+, locally advanced or metastatic cancer
- Progressed following prior hormonal therapy

- Eliminated mainly through hepatic clearance
- Uptake by OATP1B1/3
- Metabolism by UGTs
- Unique human metabolite found in clinical samples
- C14 hADME study planned

3x3 design

Chemical synthesis consideration for GDC-0810:



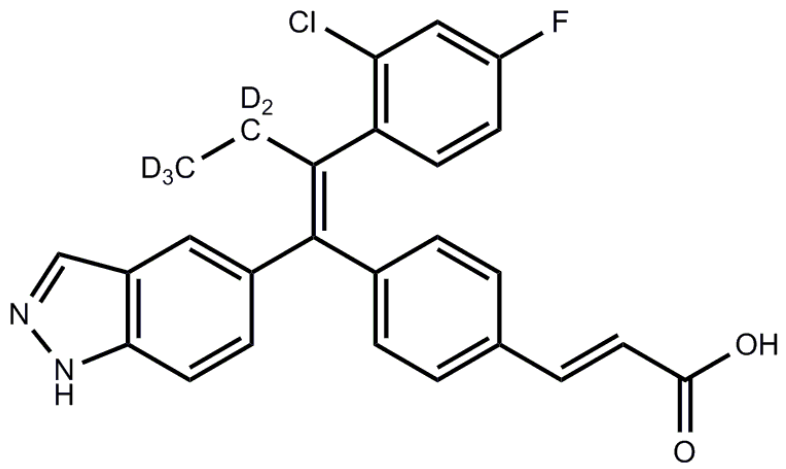
C_{max}=20μg/mL

C_0=5ng/mL

C_{trough}=5pg/mL

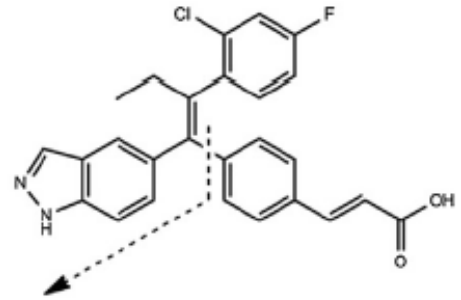
4000x diff
8-10 labels

Existing internal standard:



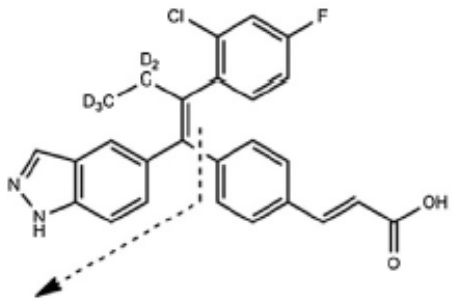
GDC-0810 labeling options:

GDC-0810



m/z: 299

d_5-GDC-0810



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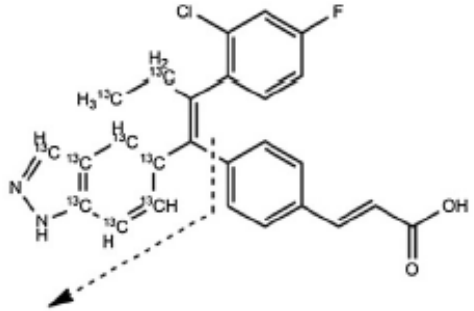
$^{13}C_9$-GDC-0810



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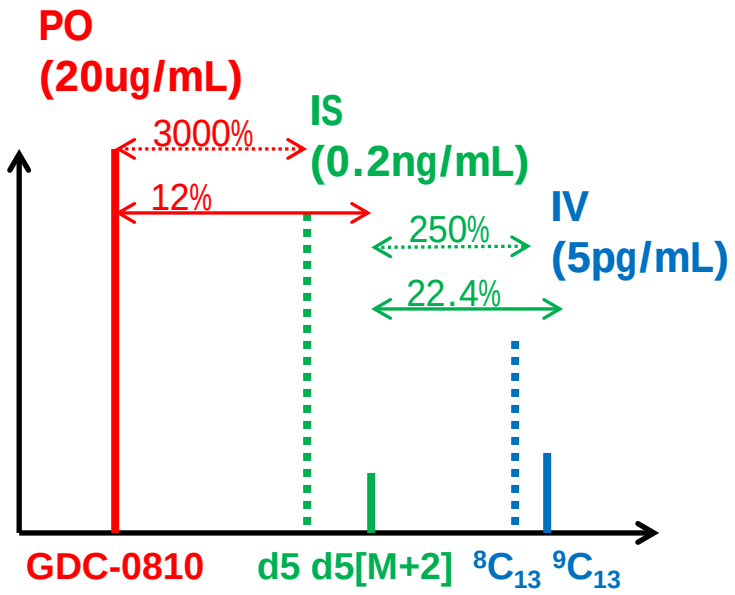


Precursor$^+$ ⟶ Fragment$^+$+Neutral loss

$[P+5]^+$ ⟶ $[F+5]^+$+N

ICSRM%=[F+5]%×[N]%

ICSRM calculation: Peak Area% of isotopic interference to the analyte (percentage of isotopic distribution if the interfering compound and analyte concentration are the same).

Compound	GDC-0810(11.4 ug/mL)	d5-GDC-0810(0.2 ng/mL)	d5-GDC-0810(2 ng/mL)
d5-GDC-0810(0.2 ng/mL) $[M+H]^+$ channel	1500(0.03)	NA	NA
d5-GDC-0810(0.2 ng/mL) $[M+H+2]^+$ channel	6(0.00004)	NA	NA
d5-GDC-0810(2 ng/mL)	150(0.03)	NA	NA
$^{13}C_8$-GDC-0810	0.002(0.000001)[a]	250(6.24)[b]	2500(6.24)[b]
[$^{13}C_9$] GDC-0810	0(0)[a]	22.4(0.56)[b]	224(0.56)[b]

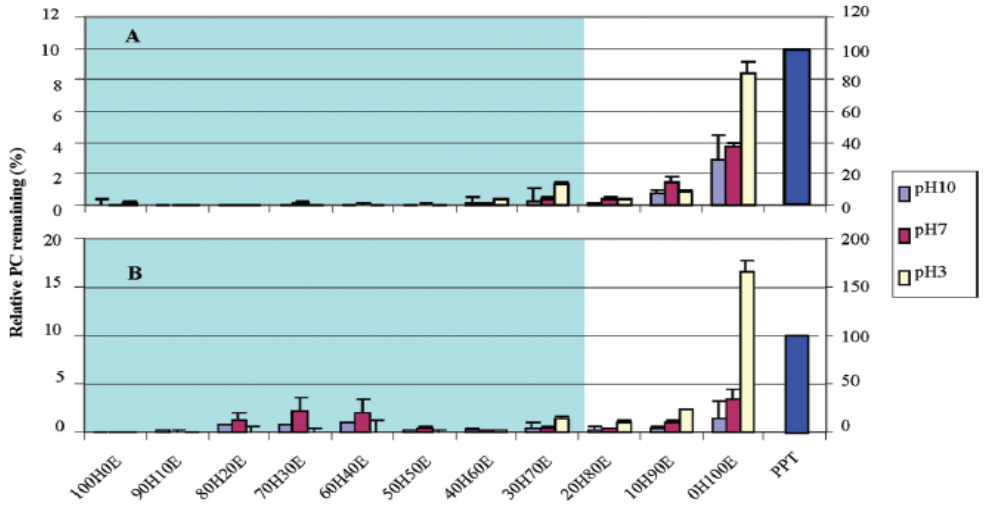
[a] Concentration of labeled IV compound at Cmax = 5 ng/mL.
[b] Concentration of labeled IV compound at Cmin = 5 pg/mL

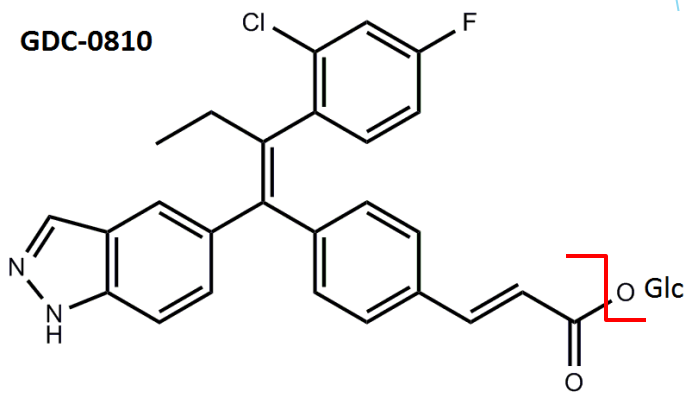
Bioanalytical considerations for GDC-0810

200uL of plasma+200uL buffer

SLE extraction by Ethyl Acetate/ Heptane (80:20)

Evaporate and Reconstitute with 100uL of buffer





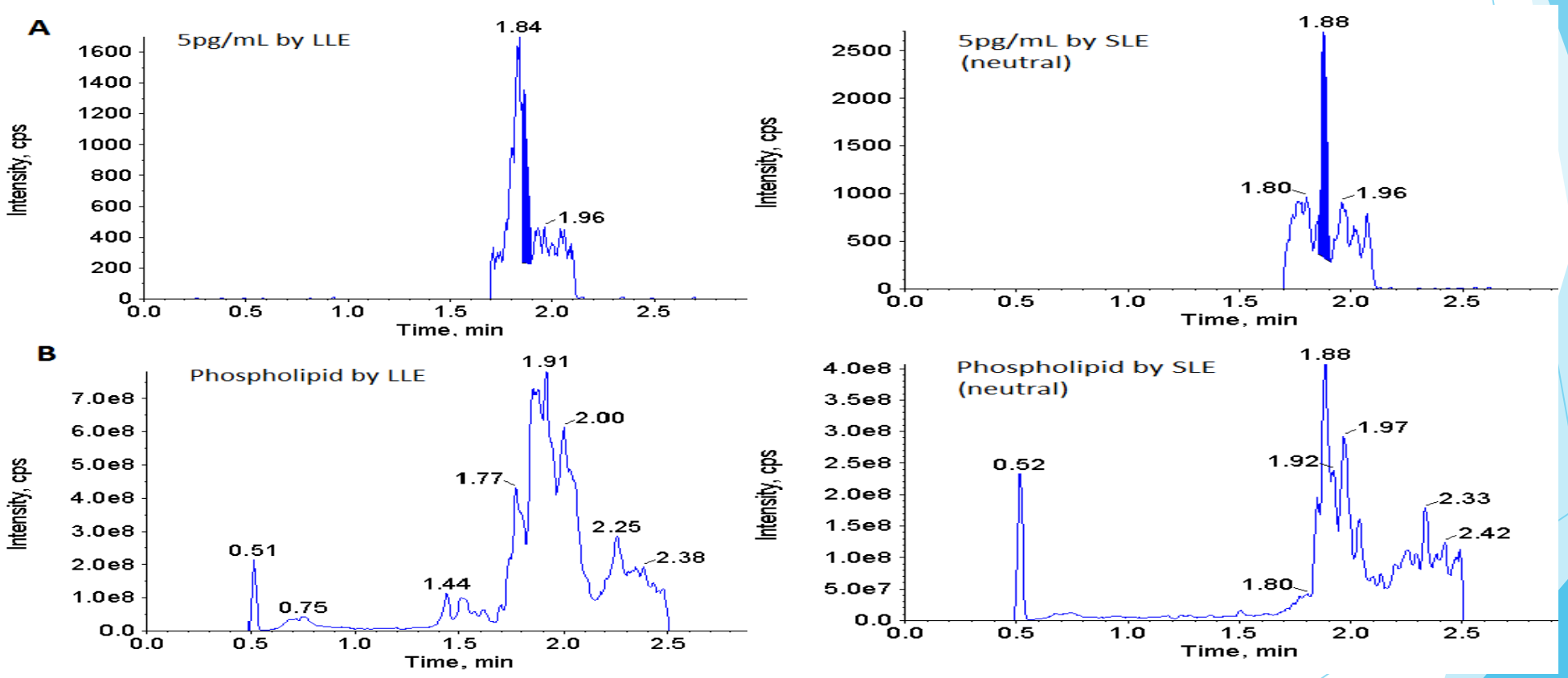
O-Gluc back conversion to 810

Ion suppression by phospholipid

Extraction pH low to high ⟶

Achieving desired sensitivity: from 5ng/mL to 5pg/mL

- Neutral buffer and solid support help to remove phospholipid and minimize ion suppression

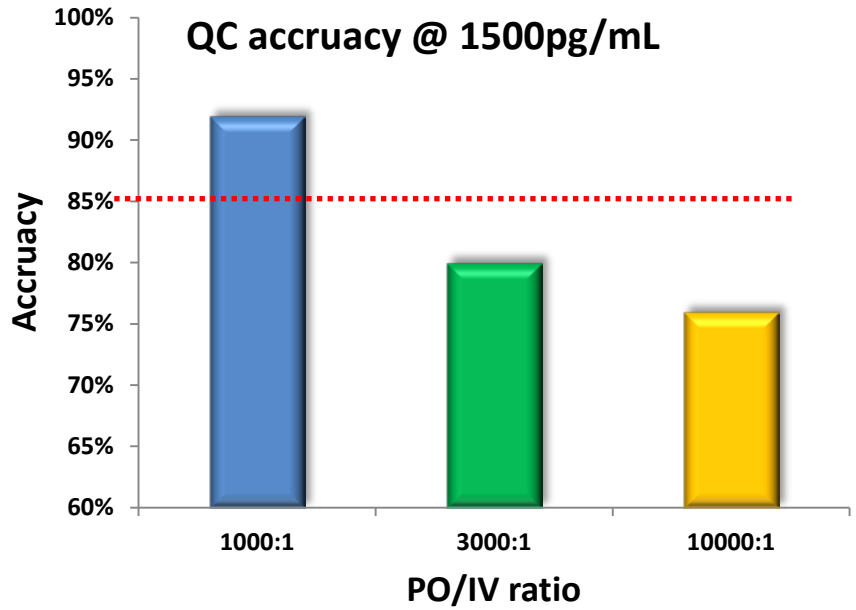


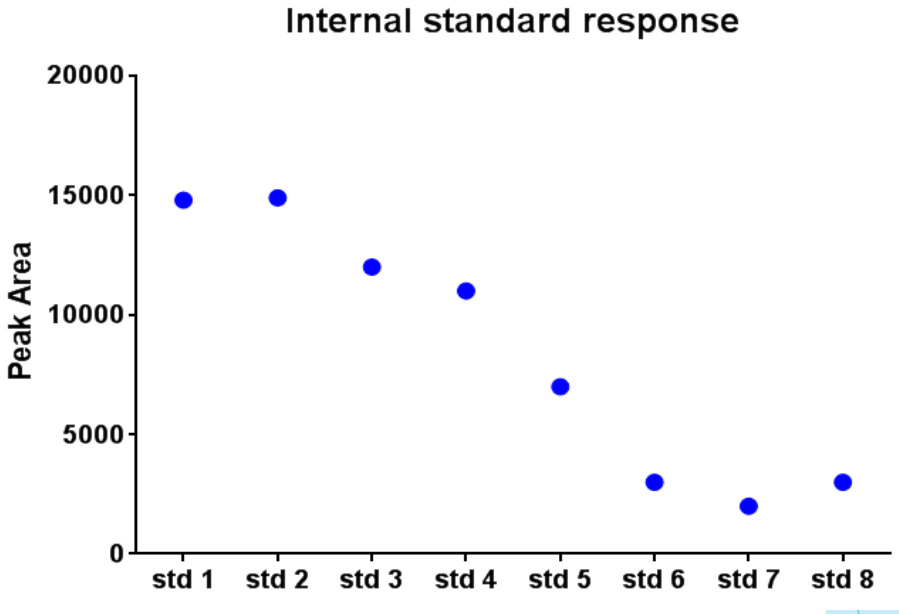
Bioanalytcial consideration: Dynamic range

- MS detector range: 10e6;
- Typical BA method range: 10e3;
- Limit for IV/PO combo method: C_{po}/C_{iv}<10e3

Compound	Oral dose	IV dose	Number of labels	$C_{p.o.}/C_{i.v.}$
Tofogliflozin	20mg	0.1mg	6	109
Daclatasvir	60mg	0.1mg	6	59
Evacetrapib	130mg	0.175mg	8	298
Ibrutinib	560mg	0.1mg	6	5.29
Beclabuvir	150mg	0.1mg	6	1000
GDC-0810	600mg	0.1mg	9	4000 (expected)

Mutual suppression at high PO/IV ratio





- Initial range 5-2500pg/mL
- Truncated dynamic range, ULOQ= 1250pg/mL
- Separate analysis of PO and IV PK

Validated method for monkey aBA study:

- Dynamic range: 5-1250pg/mL

		LLOQ (5pg/mL)	LQC (15pg/mL)	LMQC (80pg/mL)	MQC (500pg/mL)	HQC (960pg/mL)	DQC(x10) (2.5ng/mL)	HDQC(x100) (25ng/mL)
Intra-day (n=6)	%Bias	-9.7	-4.0	-10.3	6.8	6.9	6.3	-4.4
	%RSD	8.5	9.2	2.8	4.0	3.2	3.3	4.7
Inter-day (n=18)	%Bias	-1.8	0.4	-0.2	0.8	1.7		
	%RSD	11.0	8.3	10.6	7.6	7.1		

- PO/IV ratio tolerance: 1000:1 to 10000:1

QC type	LQC(1:1000)	HQC(1:1000)	LQC(1:100000)	HQC(1:100000)
%Bias	-2.00	7.19	1.33	-5.52
%RSD	6.59	3.29	11.47	4.65

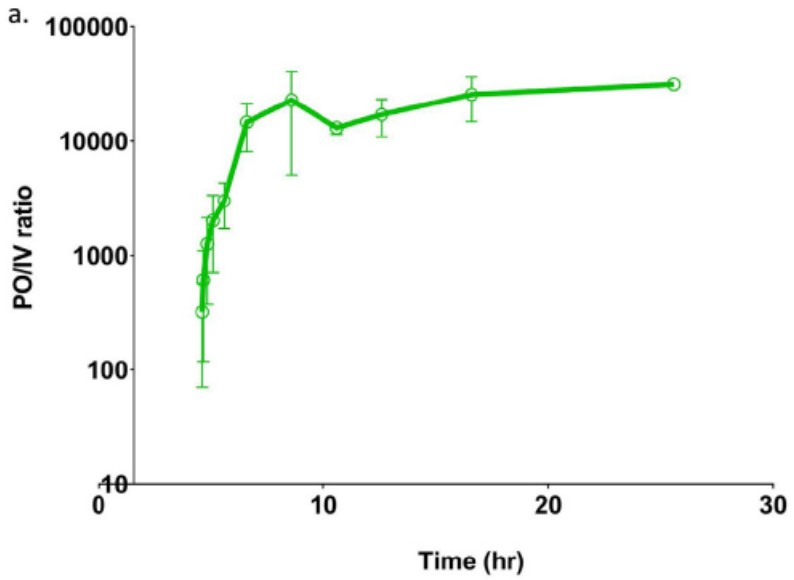
monkey aBA study design:

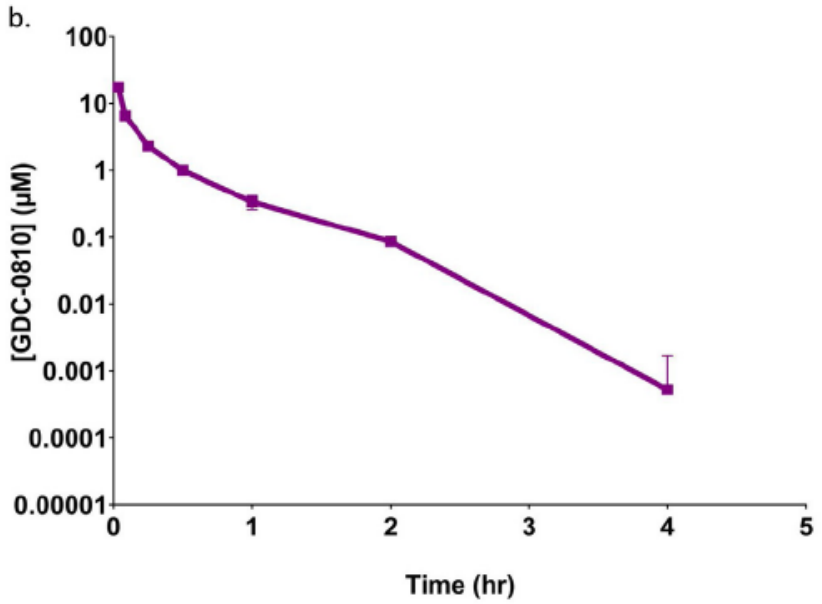
Phase I: 1mpk IV
GDC-0810

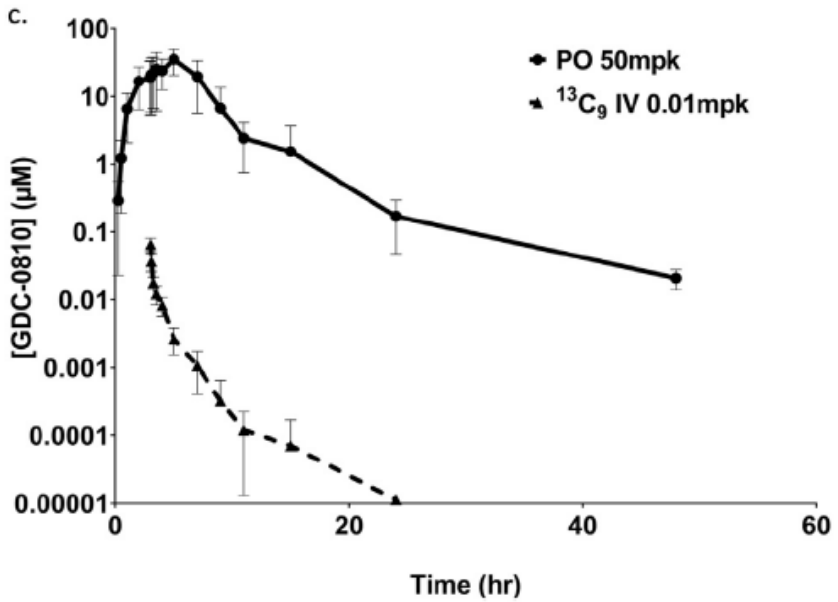
Phase II: 50mpk
P.O. GDC-0810+
0.01mpk I.V.
[$^{13}C_9$]GDC-0810

Wash out

Post-study analytical assessment:







- Retrospective evaluation: PO/IV as high as 50,000
- Additional QC(RAQC) to ensure quantitation accuracy

Analyte/IS peak area ratio RAQC Conc(pg/mL)	PO/IV ratio at 3000:1	high PO/IV ratio(ratio)	Deviation from 3000:1
50	1.25±0.08	1.19±0.02(50000:1)	-4.5%
70	1.63±0.04	1.55±0.05(25000:1)	-5.3%

PK analysis result

PK Parameters	Standard, IV (1 mg/kg)	Microdose, IV (0.01 mg/kg)	Oral (50 mg/kg)
CL (mL/min/kg)	12.6 ± 1.1	13.5 ± 3.3	-
$t_{1/2}$ (hr)	0.436 ± 0.024	1.84 ± 1.21	5.15 ± 1.55
AUC_{inf} (mM.h)	2.99 ± 0.27	0.0284 ± 0.0069	183 ± 83
C_{max} (mM)	-	-	39.7 ± 15.8
F (%)[a]	122 (58-200)	129; (82-207)	122 ± 55

- Cl, AUC/D and F% similar for standard IV and microdose IV
- Half life longer for Microdose IV possible due to highly sensitive assay
- Bioavailability higher than 100% with large variation between subjects

Conclusion:

Plan Early

- Use human dose prediction to determine label numbers
- Integrating labeling strategy with IS before GLP tox (up to 6month of synthesis of GMP material

Assess risk

- Sensitivity, stability, dynamic range, possibility of analyzing PO and IV concurrently
- PK variability, time to dose microtracer
- Availability of a second isotope labeled internal standard

Handle outcome properly

- Retrospective analysis to ensure assay coverage
- Additional validation step to cover outliers