Validation of Low Energy Accelerator Mass Spectrometer and Opportunities to Increase Industry Utilization

Stephen English, Director ^{14}C Bioanalysis

Pharmaron (Germantown) Lab Services, Inc.

Laboratory Services

Chemistry, Manufacturing and Control

Clinical Development

Biologics & CGT

Overview

- Low Energy AMS qualification
  - Comparison to LSC technology
  - Accuracy and precision at modern background
  - Linearity and co-equivalence with SSAMS
  - Evaluation of memory effects
  - Computer system validation efforts
- Leveraging LEA benefits to increase AMS usage
  - Benefits to hAME study designs
  - Benefits to ABA study designs

康龙化成
PHARMARON

Accelerator Mass Spectrometry Technologies (SSAMS & LEA)

AMS & LSC Comparison

	Graphite AMS	CO_2 AMS	LSC
Counting method	Atom counting		Decay counting
Sample preparation	Graphitization (generation of CO_2)	Direct analysis (conversion to CO_2)	Direct analysis or oxidation / solubilization
Sample throughput per week per system	~1600	~1,000	~1,000
Typical LLOQ	~0.0005 – 0.4 dpm / mL (matrix dependent)	~0.002 – 0.4 dpm / mL (matrix dependent)	20-100 dpm / mL
Data generated	pMC (percent Modern Carbon)		cpm or dpm (counts or disintegrations per min)
Preparation time (per sample)	~36 h	~15 min	~15 min

Graphite AMS
(NEC SSAMS)

CO_2 AMS
(Ionplus LEA)

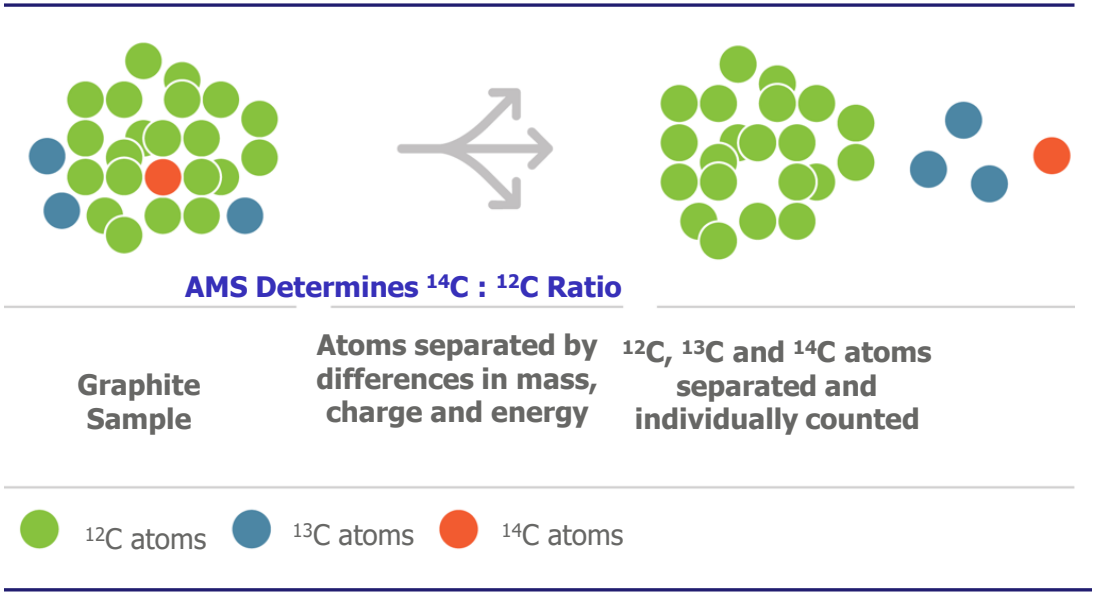
LSC

AMS and LSC are co-calibrated

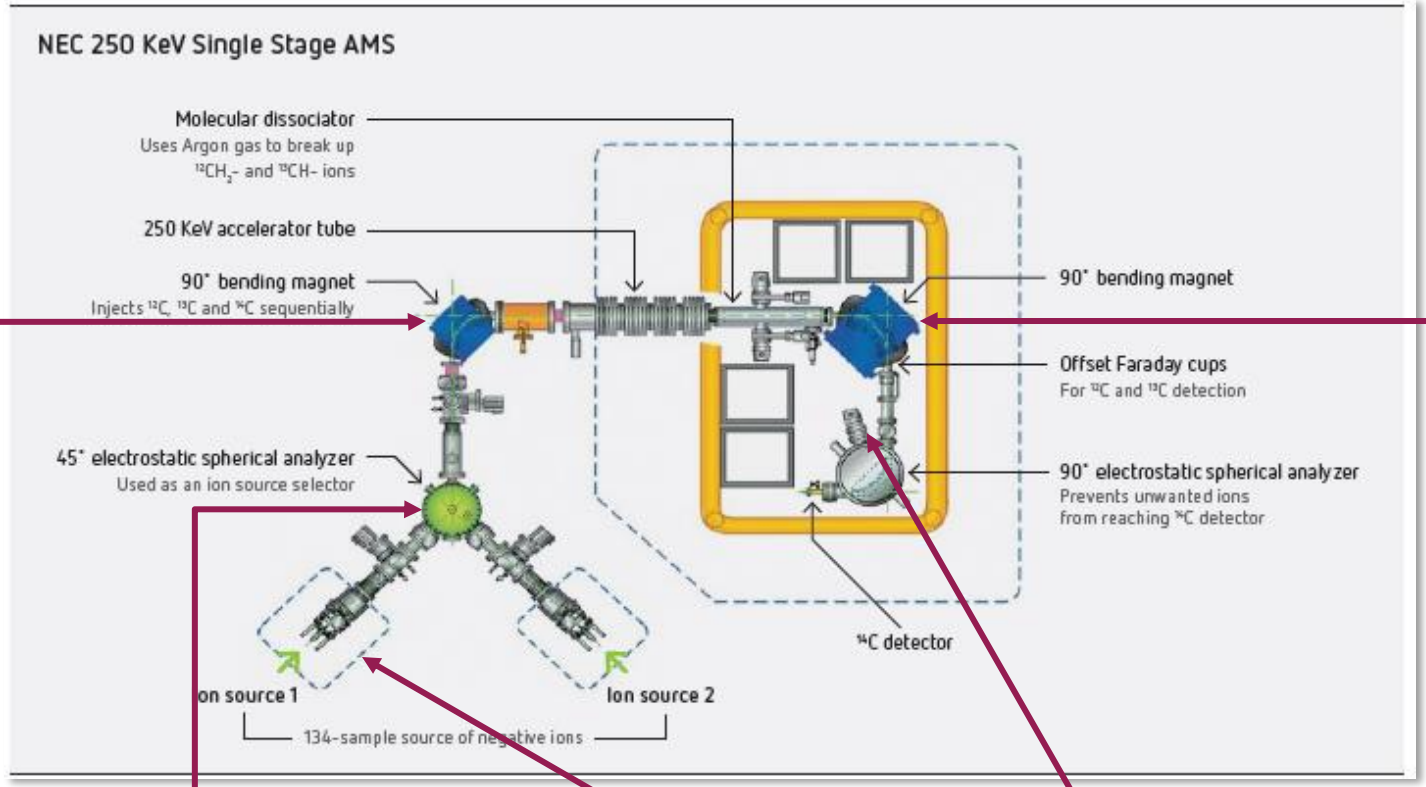
Accelerator Mass Spectrometer (AMS)

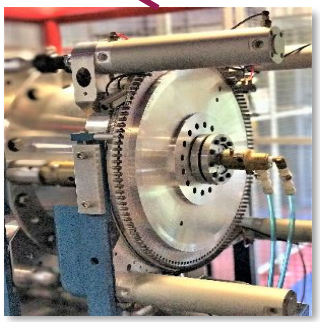
Graphite samples are subjected to particle acceleration, separated and counted at the atomic level, and as a result:

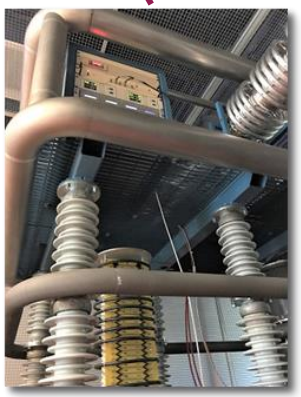
- >500 x more sensitive than decay counting technology (LSC)
- >100-1000x more sensitive than LC-MS/MS platforms

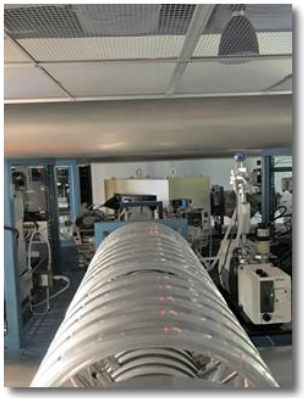


Single Stage-AMS (SSAMS)





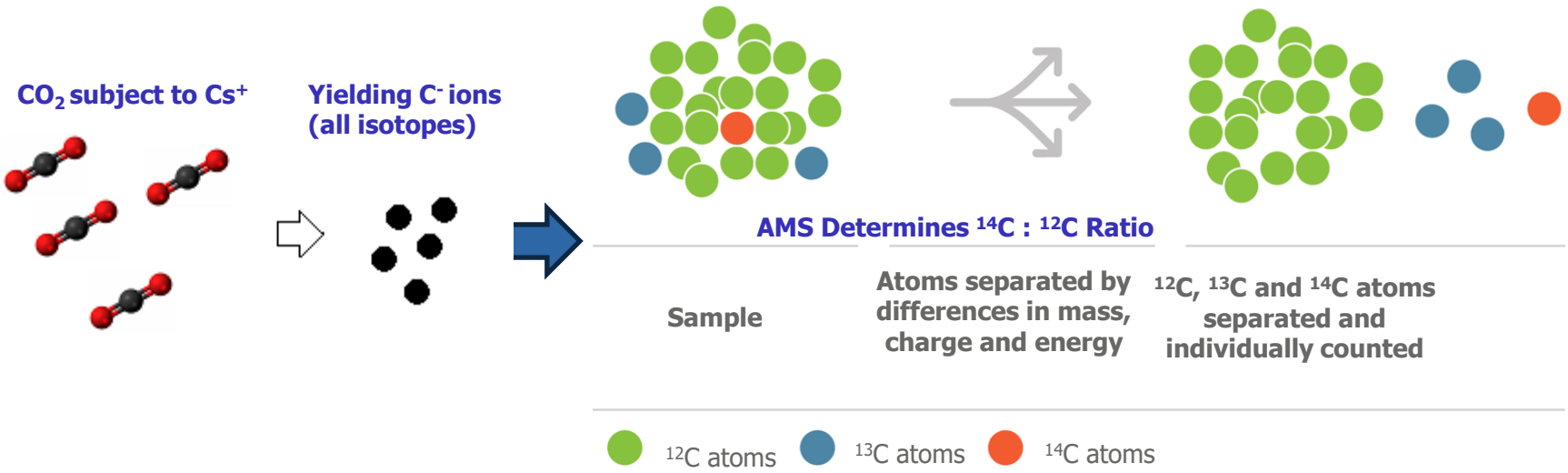




Low Energy AMS (LEA)

- Unadulterated samples loaded directly into an elemental analyzer (EA)
- Sample combusted and CO_2 is delivered to the ion source without breaking vacuum and flowed through a sample cathode
- Sample sputtered with Cs^+ ions to introduce C^- atoms into the ion beam
- C^- ions enter the 60 kV-accelerator, where, upon terminal voltage, the ions interact with stripper gas, molecular ions broken up and charge state changed from -1 to +1
- The positive ions are then analyzed by their mass and charge

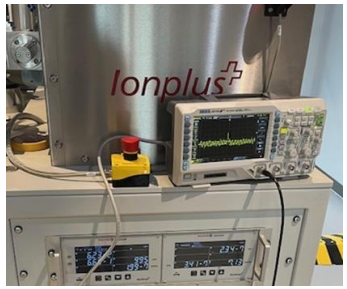
- >500x more sensitive than decay counting technology (LSC)
- Similar sensitivities to SSAMS
- No sample preparation, which accelerates analysis timelines



SSAMS and LEA Workflows

Biological Sample → Dispensed, sealed, burned with catalysts in furnace → Cryogenically transferred, converted to graphite in furnace → Graphite pressed into cathode and placed into 134 sample AMS wheel → Analyzed by AMS → Total ^{14}C data (all compound related species i.e. parent + metabolites)

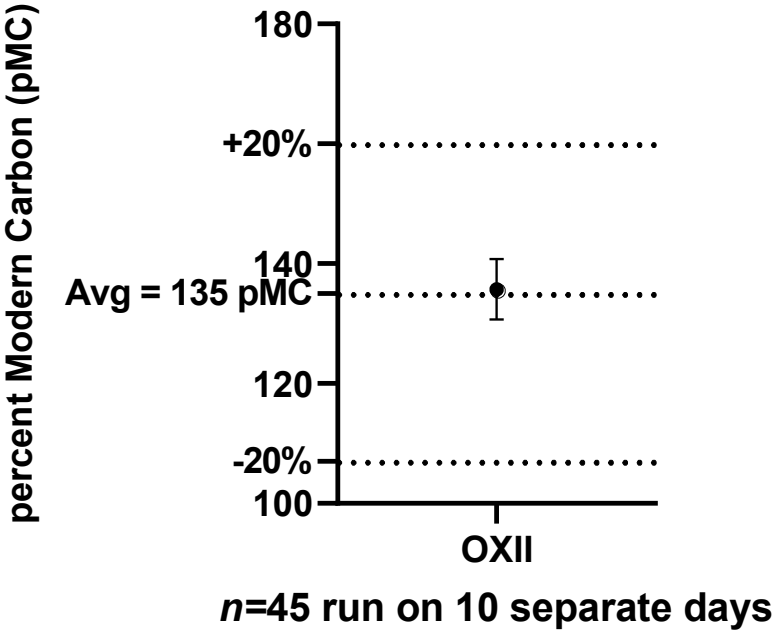
Biological Sample → Dispensed into EA tin → CO_2 introduced into ion source → Analyzed by AMS → Total ^{14}C data (all compound related species i.e. parent + metabolites)



LEA Qualification

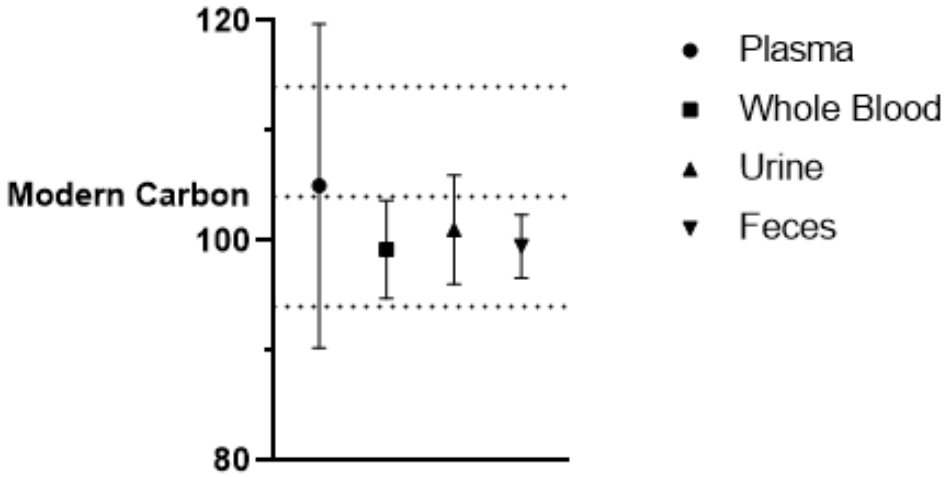
AMS Standard Accuracy and Precision

- Oxalic Acid 2, NIST Traceable, known pMC of 134 pMC
- Measurement of 45x OXII standards across 10 days
- Average 135 pMC, precision 4.7 %CV

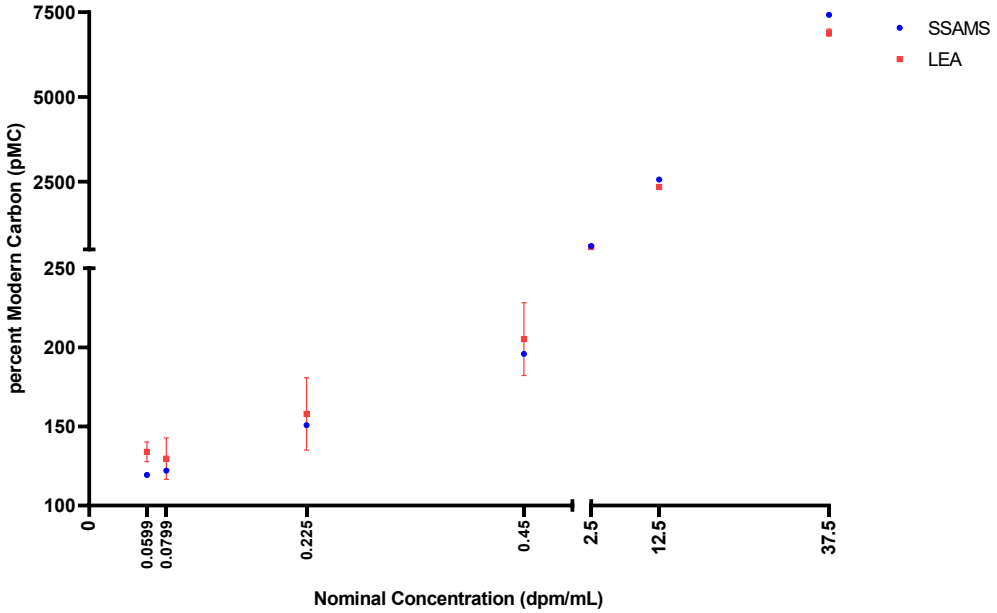


AMS Mass Balance Background Assessments

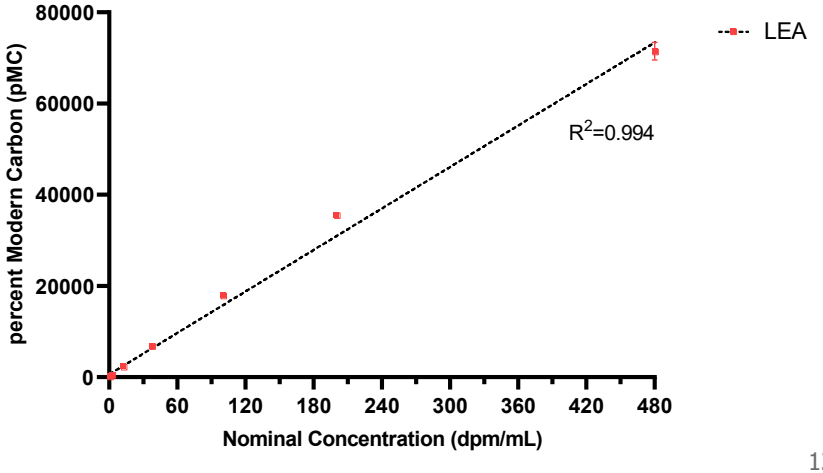
- Control plasma, blood, urine, and feces homogenate measured for pMC (*n*=20) comparing against modern carbon (~104 pMC)
- Carbon content precision: 2.12% (Urine), 5.40% (Plasma), 4.6% (Blood), 6.0% (Feces Homogenate)



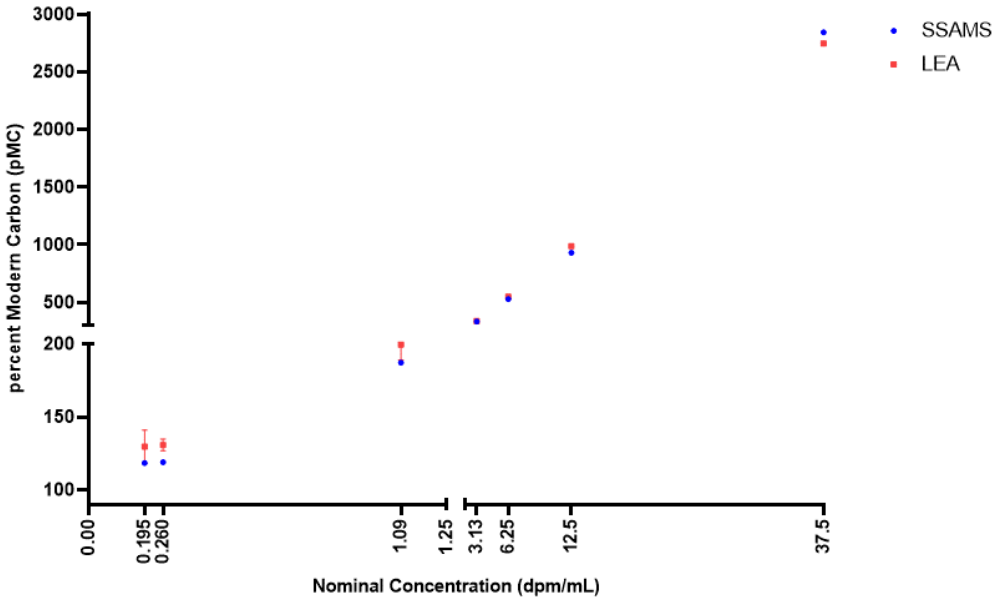
Plasma LEA Regression Lines & SSAMS Co-Equivalence



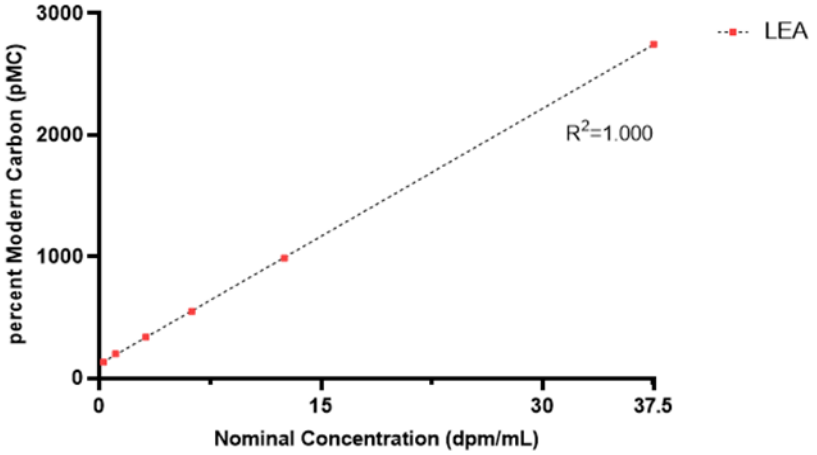
-Plasma evaluated over full equivalent dynamic range of SSAMS (up to 2.4 dpm on detector)
-Subsequent matrices limited range to approximately 5,000 pMC to mitigate memory effects
-Plasma analysis - 5 µL
-Plasma carbon content: 4.7% (5.7% CV) within 10% of Flashsmart



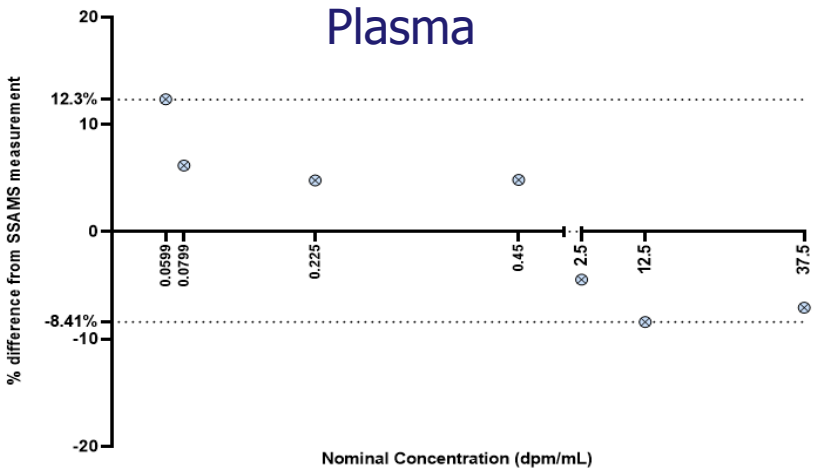
Whole Blood LEA Regression Lines & SSAMS Co-Equivalence

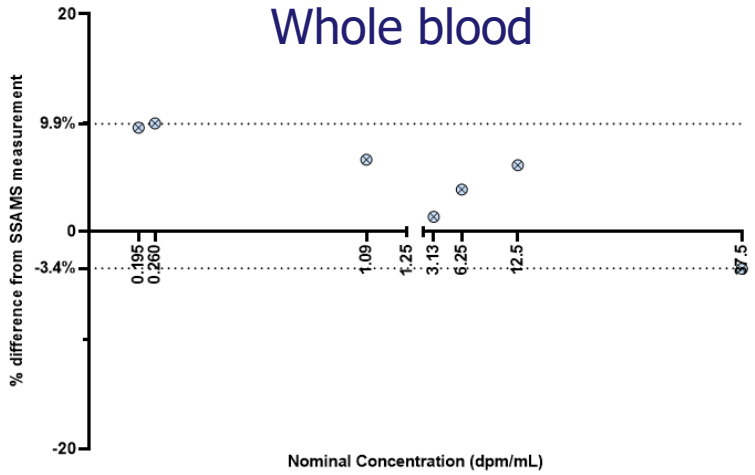


-Whole blood analysis - 5 µL
-WB carbon content: 12.8% (3.8% CV) within 15% of Flashsmart



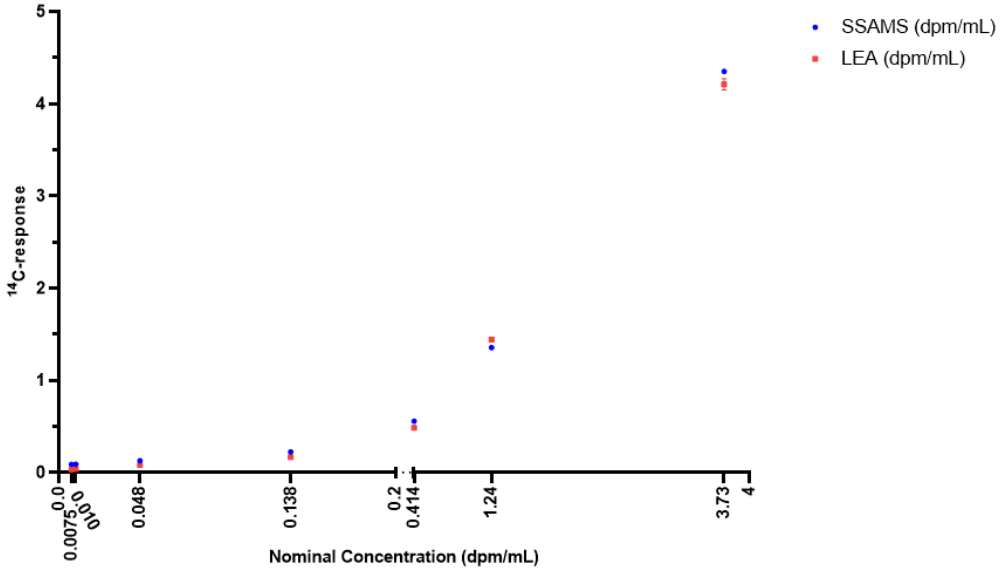
Plasma & Whole Blood SSAMS Comparison Data



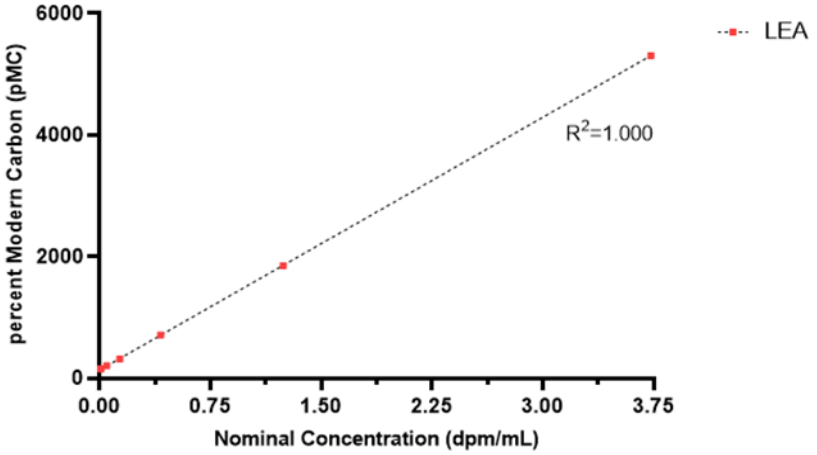


-Limit of dynamic range of SSAMS prevented full plasma comparison (plasma aliquot size on SSAMS 50 µL)
-Plasma: All comparison points with the exception of lowest value within 10% difference from SSAMS
-Whole blood: All comparison points within 10% difference from SSAMS

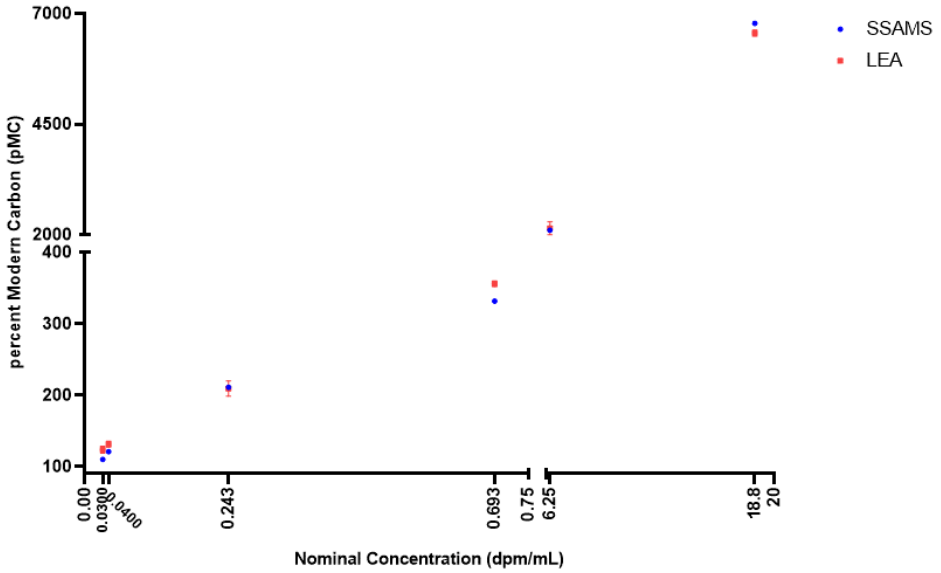
Urine LEA Regression Lines & SSAMS Co-Equivalence



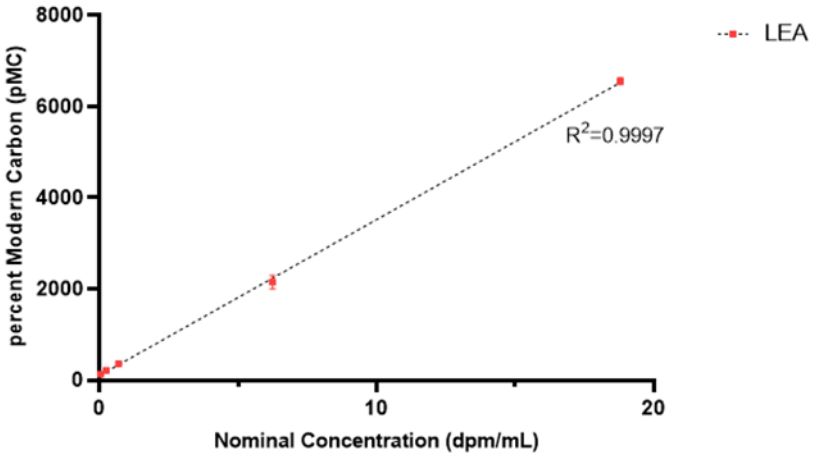
Urine analysis - 50 µL
-Urine carbon content: 0.6% (3.0% CV) within <1% of Flashsmart



Feces Homogenate LEA Regression Lines & SSAMS Co-Equivalence

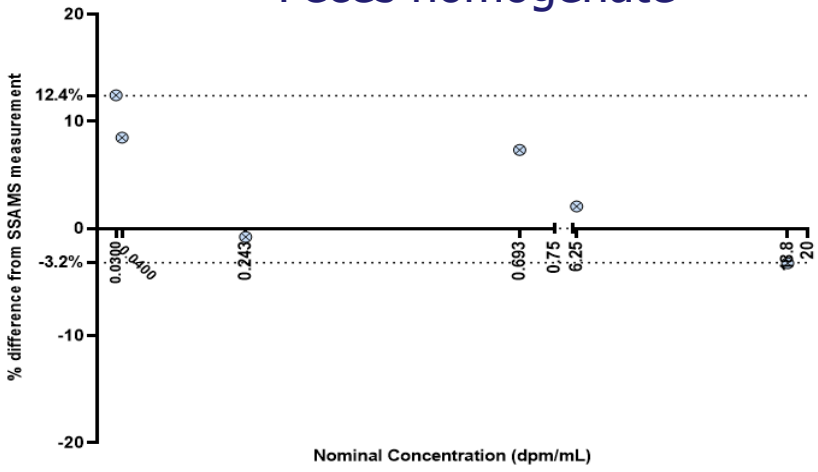


-Feces homogenate analysis – 10 mg (weighed)
-Feces homogenate carbon content: 2.5% (3.6% CV) within 2.5% of Flashsmart

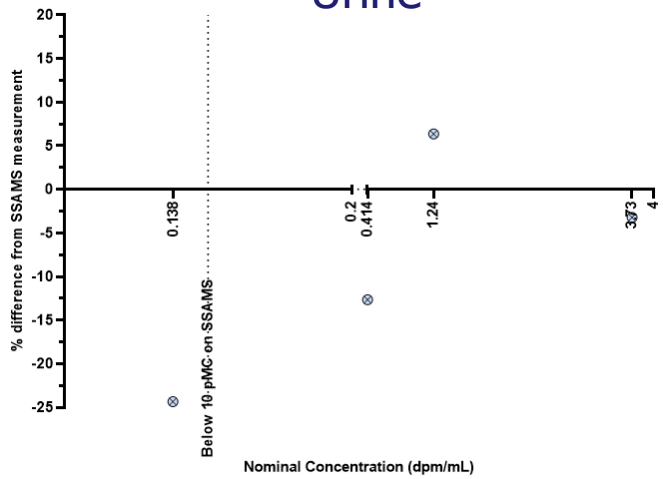


Urine & Feces SSAMS Comparison Data

Feces homogenate



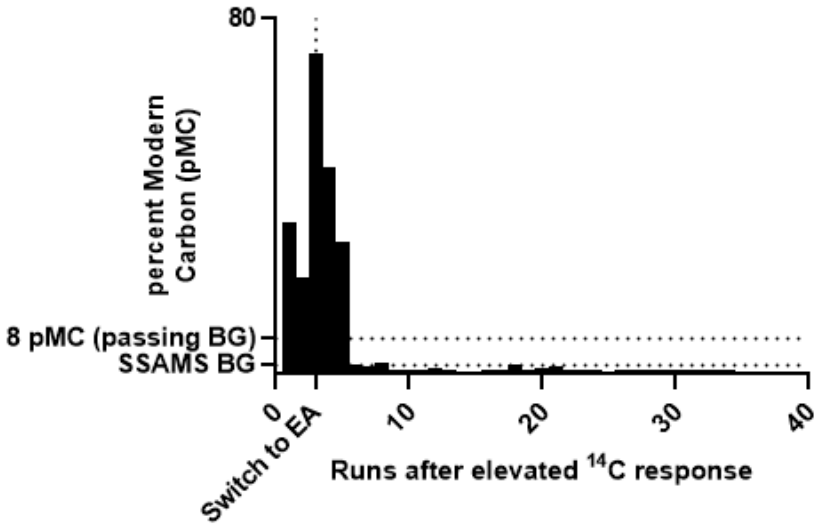
Urine



-SSAMS analysis with urine requires sodium benzoate carbon carrier and 10 µL of sample; this corresponded to the lowest 3 values below 10 pMC (being reanalyzed with more signal for better comparison)
-Feces homogenate: All comparison points with the exception of lowest value within 10% difference from SSAMS, lowest within 15%
-Urine: Comparison points with SSAMS values greater than 10 pMC within 15%

Evaluation of background following high activity sample analysis

- Bottle blank (^{14}C depleted source) – does not trigger EA
- Sodium benzoate (^{14}C depleted source) – triggers the EA
- Followed elevated ^{14}C injections (near 1 dpm in source)
- Memory effects of LEA *do* inhibit full dynamic range as compared to SSAMS



Parent analysis applications

- Parent analysis applications where a single LC fraction corresponding to eluent where the analyte of interest elutes is taken for AMS analysis
- Variables to consider:
  - Volume of fraction to AMS
  - Carbon content of sample
  - Carbon carrier utilization
  - Sensitivity as compared to SSAMS

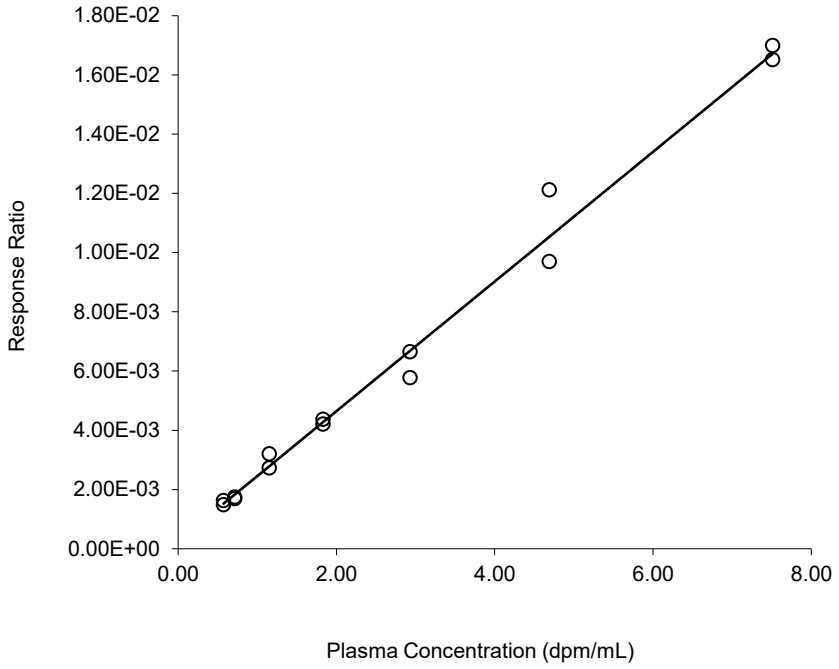
Experimental design

- uHPLC methodology utilized to optimize method run time and fraction volume
- Additional processing step introduced to fully dry LC fractions and reconstitute for equivalent volume transfer per fraction collected over the course of ~50 injections
- Dynamic range of 0.573 dpm/mL – 7.51 dpm/mL evaluated with calibration curve at start and end of batch, four QC levels (LLQC, LQC, MQC, and HQC with *n*=5 replicates)
- Conventional internal standard (^{12}C analyte) linear regression $1/x^2$ methodology utilized

Calibration curve accuracy and linearity

- All 14 calibration points (at 7 unique concentration levels) met acceptance criteria (± 15% % difference)

Calibration standard	Nominal concentration (dpm/mL)	Calculated concentration (dpm/mL) Batch 1	Accuracy (%)	n
7	0.573	0.560	103	2
		0.622		
6	0.716	0.653	92.7	2
		0.675		
5	1.15	1.34	107	2
		1.12		
4	1.83	1.80	100	2
		1.87		
3	2.93	2.52	92.8	2
		2.92		
2	4.69	4.31	104	2
		5.41		
1	7.51	7.42	100	2
		7.64		
Slope		2.19E-03		
Intercept		2.68E-04		
r^2		0.99		
n		14		



Quality control accuracy and precision

- All intra-batch acceptance criteria as defined in the ICH M10 guidance on chromatographic methodology met acceptance criteria
- All individual QCs also were within 15% difference from target

Batch number	LLQC	LQC	MQC	HQC
	0.574	1.14	2.94	4.71
	dpm/mL	dpm/mL	dpm/mL	dpm/mL
1	0.545	1.13	2.58	4.77
	0.620	1.09	3.05	4.83
	0.518	1.02	2.70	4.61
	0.564	1.18	2.81	4.90
	0.557	1.18	2.81	5.42
Intra-batch mean	0.561	1.12	2.79	4.91
Intra-batch accuracy	97.8	98.1	94.9	104
Intra-batch precision (%CV)	6.68	6.02	6.2	6.24

Parent analysis conclusions and future work

- Linearity and interpolation onto a calibration curve works as intended for LEA instrumentation
- Modifications to workflow, including the fraction dry-down step, do not impact sensitivity or accuracy or precision of methodology
- uHPLC methodologies, shortened run times, and speed of processing could position the technique in a comparable turn-around-time to conventional LC-MS/MS
- Additional work on background and signal to noise required to lower LLOQ to ~0.1 dpm/mL

Ionplus LEAMS Computer System Validation

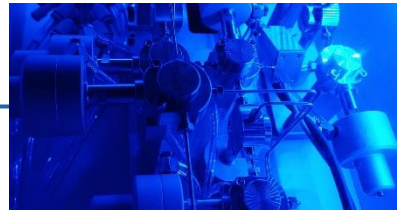
- Unlike currently available AMS instrumentation, LEA's software design is built with pharma industry data integrity considerations (EMA data integrity and 21 CFR Part 11 compliant rigor)

Vario ISOTOPE select (EA)

- Populates carbon data and calibration curve

Automated Magazine Changer (AMC)

LEA intranet system



ACS

- Controls instrument operations
- Monitor system specifications
- Gathers data on targets

HTI

- Captures EA CO_2 gas
- Modulates and maintains consistent flow and pressure for targets in the ion source

BioLMP

- Sequence & batch analysis
- Data processing
- Database for data retention

Ionplus LEAMS Computer System Validation

- Full computer system validation performed on LEA software system including:
  - Validation Project Plan
  - User Requirement Specification
  - System Design Specification
  - Electronic Records and Electronic Systems Assessment Form
  - System Risk, Impact, and Criticality Assessment Form
  - Factory and Site Acceptance Testing
  - Gap Assessment
  - Gap IOPQ Protocol
  - Gap IOPQ Protocol Executed
  - Trace Matrix
  - Validation Summary Report

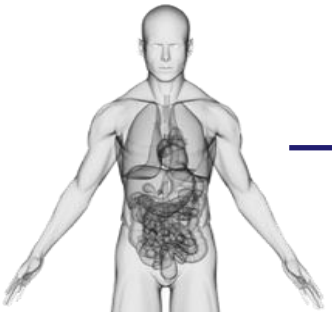
Opportunities to increase industry utilization

^{14}C hADME Typical Study Design

Therapeutic mass dose containing ^{14}C-drug via therapeutic dose route

- 1 – 5 µCi low dose (Microtracer)
- 50 – 100 µCi high dose (Conventional)

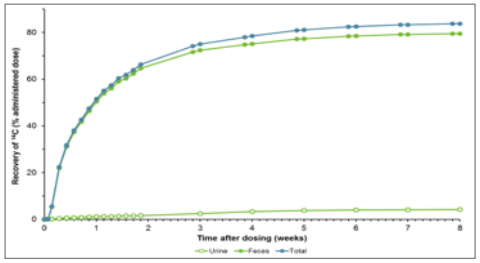
~8 Subjects



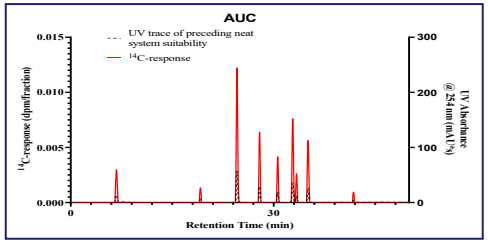
Typical study duration: 7-14 days + intermittent 24 h collections until ^{14}C excretion complete

Mass Balance Samples

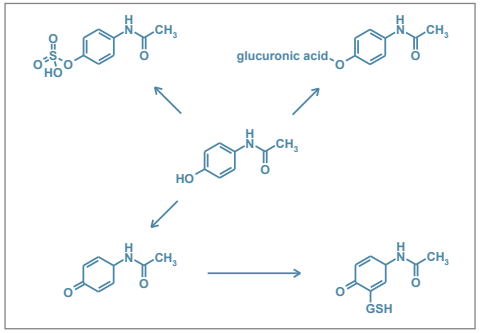
- Blood
- Plasma
- Urine
- Feces



Mass Balance



Metabolite Profiling



Metabolite Identification

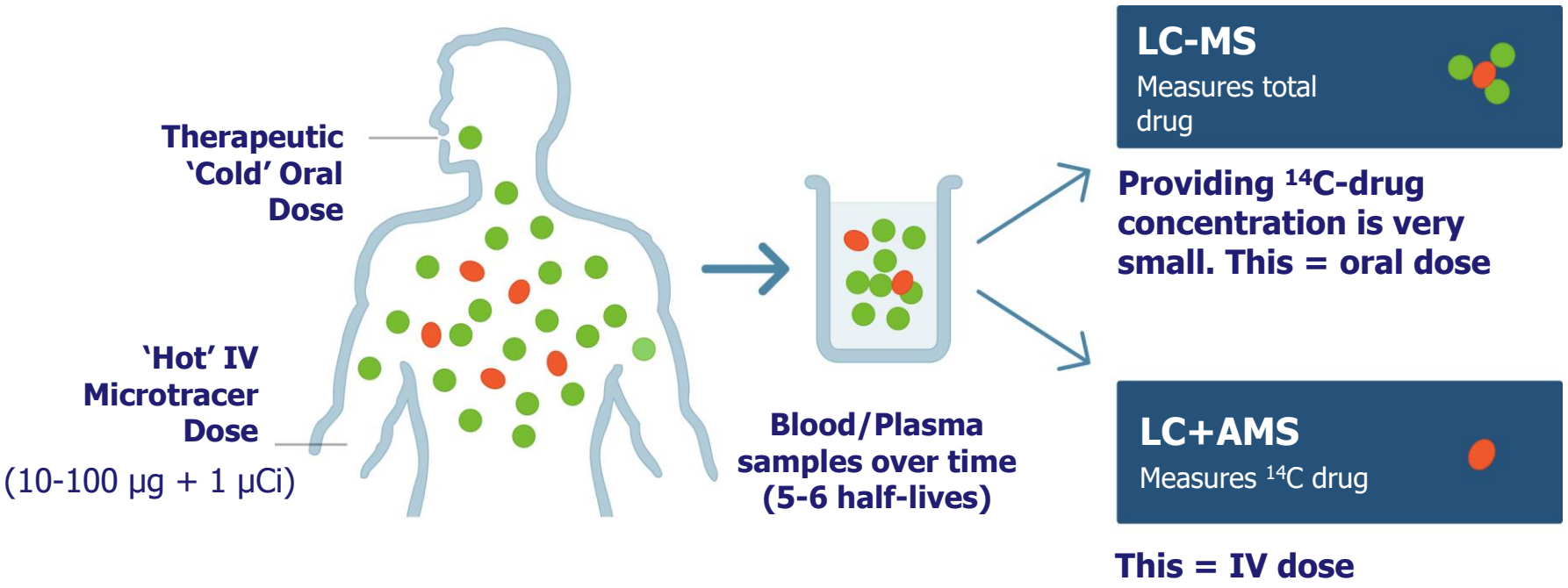
LEA benefits to hAME

Study Type	When?	Typical ^{14}C dose	Dosimetry assessments required	Typical ^{14}C requirements	Total ^{14}C data	Metabolite profiling
Classical Macrotracer approach	• Conventional approach • Pre-clinical assessments	100 µCi	Yes	GMP	LSC	MSC
Hybrid Macrotracer with AMS	• Low F, high CL, high V_d compounds	100 µCi	Yes	GMP	LSC & AMS	MSC and LC+AMS
Microtracer approach	• Restricted dose (toxicity) • Long $t_{1/2}$ • Accelerate development • Small quantity of ^{14}C • Unstable ^{14}C / insoluble • Sensitive populations	1-5 µCi	Sometimes	Non-GMP	LSC & AMS	LC+AMS

-With less than or equivalent turnaround time to LSC, LEA will support real-time release criteria for mass balance applications of microtracer approaches

-Extraction efficiency development optimization increased with rapid turnaround of changing extraction procedures (1-2 week speed increase for metabolite profiling development)

^{14}C hABA Typical Study Design



- No IV safety data required
  (Oral tox package sufficient)
  Full GMP manufacture not required
  Minimal IV formulation effort
  - IV dose 10-100 µg
- Reduced clinical costs
  - Single dosing occasion
- Optimal scientific design
  - Avoids issues with dose-dependent kinetics
  - Within subject absolute bioavailability

Referenced in FDA Mass Balance Guidance (July 2024)

Information on the absolute bioavailability of the investigational drug can help interpret mass balance data and understand the overall drug elimination pathways.

When only the oral formulation is being developed, an absolute bioavailability study can be combined with the mass balance study in a single protocol in a two-part study. One part can be the human radiolabeled mass balance study for the orally administered investigational drug (mixture of non-radiolabeled and radiolabeled doses). The other part can determine the absolute bioavailability of the investigational drug administered as an oral non-radiolabeled dose (see

Guidance for Industry (fda.gov)

Conclusions

- Accelerator Mass Spectrometry has been utilized in drug development for over two decades as an ultra-sensitive detection technique for radiocarbon
- Low Energy AMS has been qualified and is determined to be a co-equivalent detector to graphite SSAMS for all applications types
- Low Energy AMS leverages speed of analysis to render fast data turnaround times
- 2024 FDA Mass Balance Guidance offers an opportunity to navigate human AME and ABA studies with reliance on all available technology
- LEA represents an opportunity for the industry to support more microtracer applications in clinical development with equivalent data accuracy, precision with faster turnaround times

Acknowledgements

- Dr. Marie Croft – Scientific Director, Radiolabeled Sciences
- Germantown instrumentation team
  - Chris Tilley
  - Benjamin Miller
  - Carter Taliaferro
  - Justin Houck
- Germantown leadership team for support
  - Mitesh Sanghvi, Ph.D.
  - Joana Oduro, QA
- Sarah Bennett & Uma Ranganadham (Computer System Validation)
- Adrian Pereira (Pharmaron UK AMS team)
- Graeme Young, Ph.D.