

Overview

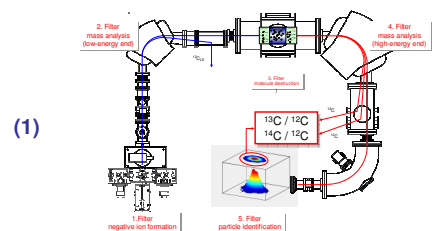
Mass Spectrometry and other forms of proportional quantitation enlist calibrations plots with independently placed QCs for Accuracy and Precision. Similar expectations are being asked of Accelerator Mass Spectrometry in Absolute Bioavailability investigations (3). Isotope ratio (¹⁴C/C) analysis by AMS however provides linear and absolute quantitation (2) after the conversion of all organic materials to an inorganic matrix (graphite) (8). Calibration plots serve principally to correct for procedural losses in the extraction and LC fraction collection process prior to AMS.

Isotope-labeled drug recovery from the matrix is thus the key variable that controls the final A/P of the method. Fortuitously, unlabeled carrier analyte can be added to create a single concentration extraction method that mitigates the confounding effects of concentration-dependent recovery.

We analyzed data from "traditional" calibration plots used over the course of a multi-batch study where recovery had been optimized using a rigorous incubation with unlabeled carrier prior to extraction by protein precipitation. The data suggests that any slope/intercept combination from the three plots would lead to passing results as reported by QC for accuracy and precision. It also shows that a single calibration level (recovery correction standard) can be used in place of multi-point plots to achieve similar results. Our statistical approach to LLOQ determination is also presented and shown to be equivalent to the traditional approach (%CV at Lowest Standard).

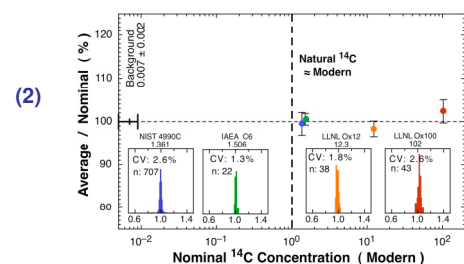
Isotope Ratio AMS Process is Inherently Linear

Accelerator Mass Spectrometry (1) traces low abundance carbon-14 for high specificity, but detects them with mass spectrometry for high sensitivity. Normalization of the ¹⁴C response to a common stable isotope beam normalizes any variability in the ion source ionization and ion beam transmission (1).



(1)

System suitability standards analyzed over the course of many months are shown in (2). These are only "checks" used for analytical assurance as they can only "fail" with gross machine malfunction (rare to never).



(2)

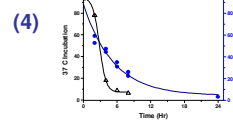
Absolute BA Study Parameters

- 250 nCi (40 µg) i.v. dose administered concomitantly with 5 mg oral dose (3)
- LC-MS/MS method development indicated potential for poor extraction recovery at low analyte concentrations (i.e. <0.5 ng/mL) due to high affinity receptor
- An optimized extraction method with predictable and concentration-independent recovery was developed using incubation with excess unlabeled analyte
- Multi-point calibration plots were attached to each set of sample batches

Optimize Extraction Method: Displace ¹⁴C Analyte with Incubation

High and Consistent Recovery is critical to reproducible results. Bound labeled drug can be displaced via cold drug incubation

- Incubate ¹⁴C spiked plasma with unlabeled drug, incubate at 37°C to allow interaction with receptor and freeze
- Incubate at ambient and 37°C
- Precipitate proteins by acetonitrile (2X)
- Measure washed pellets for residual ¹⁴C by AMS
- 4 hr at 37°C provides >90% recovery
- At ambient, 24 hrs would be required (too long for viable method)



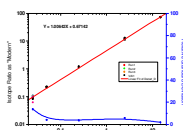
These experiments were done for 24 hours at room temperature using unlabeled drug as a carrier (1.8 µg/mL, fortified), and for eight hours at 37°C. The primary analysis was based on carbon-14 remaining in the protein pellet. At physiological temperature of 37°C, the extraction occurs much more quickly and displays a cooperative? release profile.

(4)

Standards and QCs for Batch Analysis

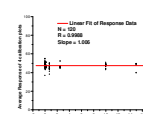
- Standards (5) and QCs (LLOQ, Low, Mid, High in quadruplicate) prepared in plasma, incubated and then frozen to mimic study samples (in vivo receptor labeling).
- Range: 0.00 to 15 dpm/mL (1.9 to 1144 pg/mL; range 600-fold; LLOQ at 0.025 dpm/mL).
- Extraction method: a 4-hr incubation (see 4) of plasma in the presence of 1.8 µg unlabeled analyte (create single concentration level for total analyte)
- Isotopomers were purified by UPLC fraction collection and analyzed by AMS after dilution into a defined carbon carrier (tributyrin, 1 µL, see 8)).
- Accuracy/Precision (A/P) data reported by QCs
- Acceptance Criteria: Acc. 85-115%; Precision +/- 15%
- Linear Regression Models using a floating and a fixed Y intercept approach (5).
- Average response at each level also calculated (Modern/DPM/mL) to visualized response (6)

(5)



Fitted Standard Plots from 4 runs over a 1 month time course. The highest group RSD was 13% at the LLOQ (blue axes)

(6)



A different view: Average response (MC/DPM/mL) at Standard levels fitted with linear regression (slope of 1.006 indicates linearity across total standard concentration range)

Relationship between Regression Models and Single-Point Calibration for QCs

The effect of fixed Y intercept vs. floating and two weighting choice were analyzed for slope and intercept. The obtained slopes were then sequentially inputted back into the calculation sheets and the QC results recalculated.

Data showed the regression/weighting model to have no effect on the passing rate of QCs (12 QCs per run)(7). When the slope was derived from between baseline (fixed Y intercept set by tributyrin ¹⁴C level) and a single standard point (0.25 DPM/mL) - All QCs passed. This is attributed to equal analyte recoveries across all concentration levels (6).

(7)

Intercept Weighting	5 point linear regression					
	Floating		Fixed 1/Y ²		Floating None	
	Slope	QC P/F	Slope	QC P/F	Slope	QC P/F
Run1	4.775	12/12 Pass	4.606	12/12 Pass	4.775	12/12 Pass
Run2	4.466	12/12 Pass	4.434	12/12 Pass	4.466	12/12 Pass
Run3	4.616	12/12 Pass	4.785	12/12 Pass	4.616	12/12 Pass

8 healthy male subjects
~40 µg (240 nCi) [¹⁴C]drug single dose IV infusion (15 min) administered at 1 hr (Tmax) after the oral dose of drug (5 mg)

24 hr blood sampling for analyte [¹⁴C]analyte plasma PK parameter estimation

Parent drug concentration measured using two analytical methods

AUC by LC/MS/MS

AUC for IV dose determined By UPLC-AMS assay

Plasma Sample

Collect analyte fraction

Vacuum dry

Add 1 µl tributyrin

Incubate for 4 hr

Precipitate proteins

Inject on LC

Vitalea BioMICADAS AMS - compact with 24/7 operational capability

CO₂ + Zn → CO + ZnO

CO + CO → Fe, Co → CO₂ + C fullerene

500°C

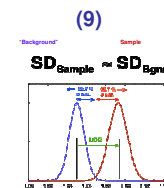
LLOQ Determination

Traditional: +/- 20%CV at LLOQ spiked standard (0.025 DPM/mL here).

Statistical: Baseline is a defined isolatable background sample (tributyrin)(8). Use signal-to-baseline approach.

AMS produces homoscedastic analysis - Equivalent variance at low concentrations.

The baseline and analyte are fully additive (9).



A multiplier of 6 represents a likelihood of only 9 parts per million that normal distributions of the background and the sample having equivalent uncertainties would overlap at the 3 sigma levels

(10)

	Conc. [dpm/pg/mL]	Conc. [amol/mL]	%CV	amol ¹⁴ C per LC fraction
LLOQ N=4	0.025 dpm/mL 1.93 pg/mL	180 amol 14C/mL plasma	7.6	6.2 from drug 15.4 from carrier
LLOQ (statistical) N=4	0.0183 dpm/mL 1.40 pg/mL	154 amol ¹⁴ C/mL plasma	3.4	15.4 amol ¹⁴ C from carrier

The LLOQ values (10) are in close agreement as the 0.025 dpm/mL QC represents 7.2 amol of ¹⁴C analyzed over a 25 amol ¹⁴C/C tributyrin baseline, close to the limit for the 6 X SD approach (9). The scatter in the traditional approach was 7.6%CV, well below the 20% limit in FDA Bioanalytical Guidance Document

Conclusion

With sources of non-linearities controlled by instrument (IR-AMS) and within method (optimized extraction with cold carrier) a single point calibrant can provide accurate results.

Acknowledgements

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