

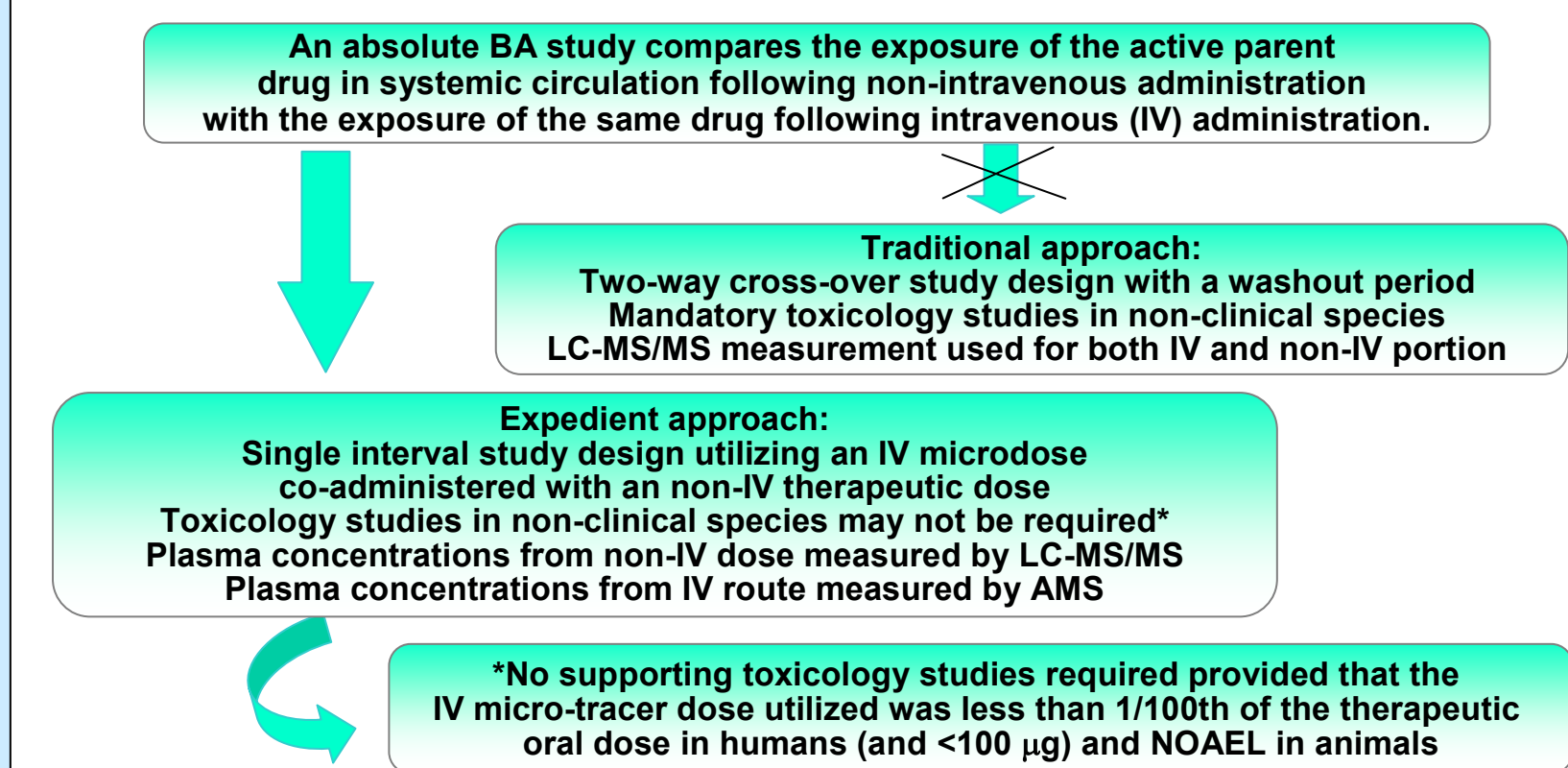
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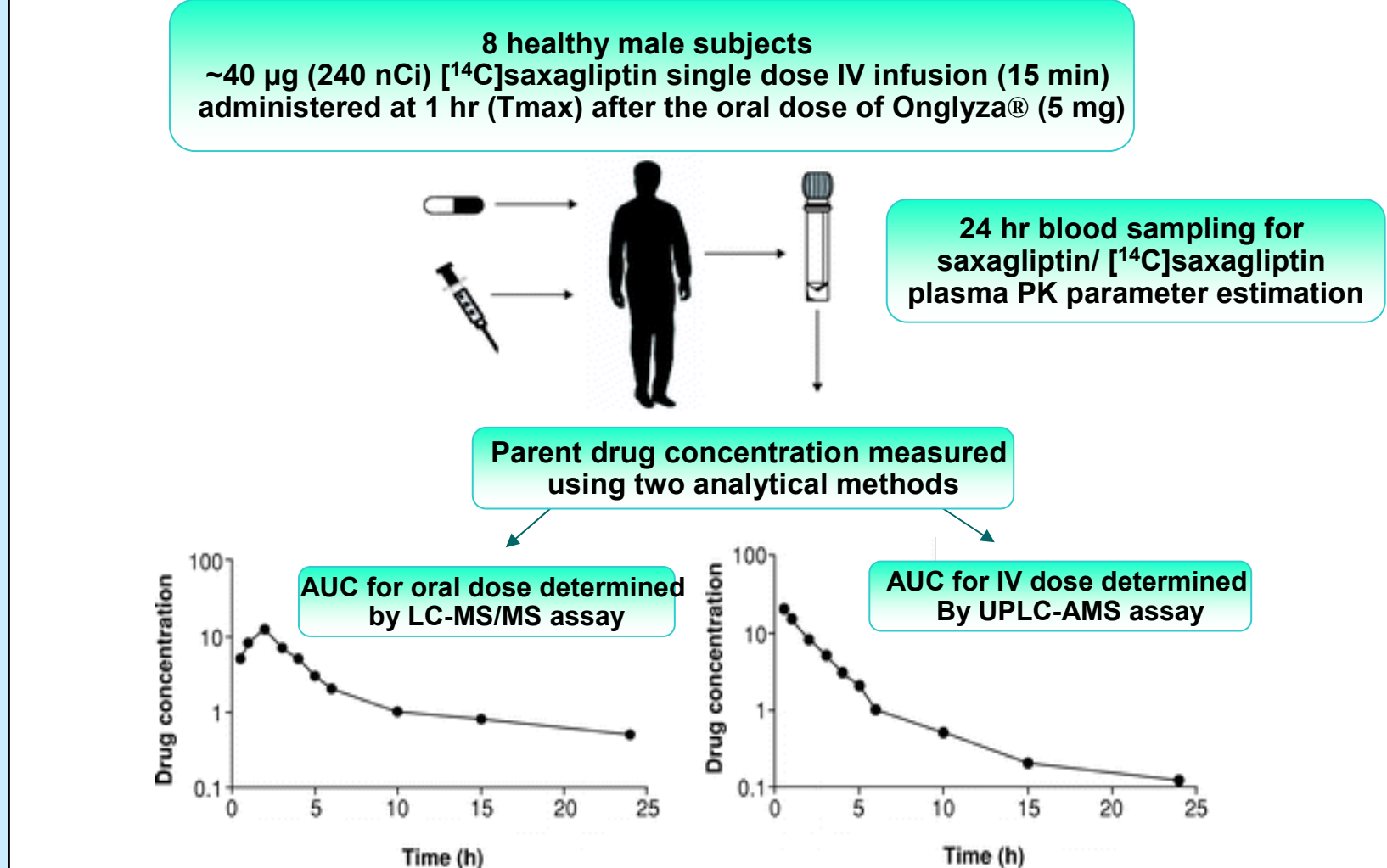
Introduction

- Onglyza® (saxagliptin, BMS-477118) is an oral, small molecule DPP4 inhibitor, indicated for the treatment for Type II diabetes.
- A clinical absolute bioavailability (BA) study was required to fully understand the disposition of saxagliptin.
 - The clinical study design included concurrent IV administration of a [¹⁴C]saxagliptin microdose (40 µg, 240 nCi) at the projected T_{max} of a 5 mg therapeutic oral dose.
 - The analytical plan involved analyzing plasma concentrations of [¹⁴C]saxagliptin derived from the IV route of administration by AMS (accelerator mass spectrometry) and unlabeled saxagliptin from the oral dose with a validated LC-MS/MS method.
- LC-MS/MS method development indicated the potential for poor extraction recovery at low saxagliptin concentrations (i.e. <0.5 ng/mL).
- The human ADME study showed that saxagliptin and 5-hydroxysaxagliptin (an active metabolite) were the major drug-related components in the circulation along with several minor metabolites.
 - Chromatographic separation of saxagliptin from its metabolites was required for AMS analysis of [¹⁴C]saxagliptin
- After overcoming the analytical challenges, the method was successfully validated and was applied to the quantification of [¹⁴C]saxagliptin in human plasma from an absolute BA study.

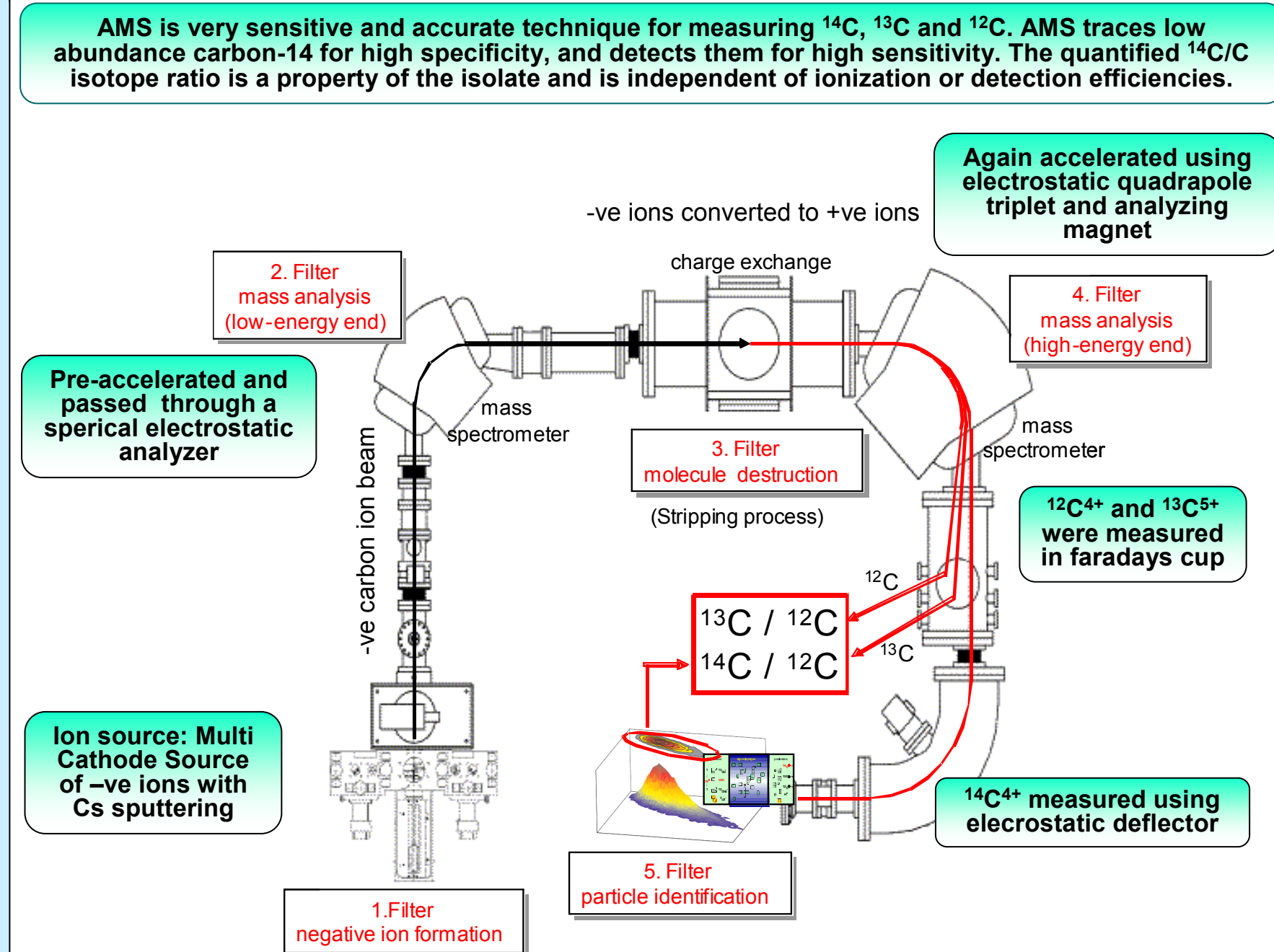
Absolute BA Study



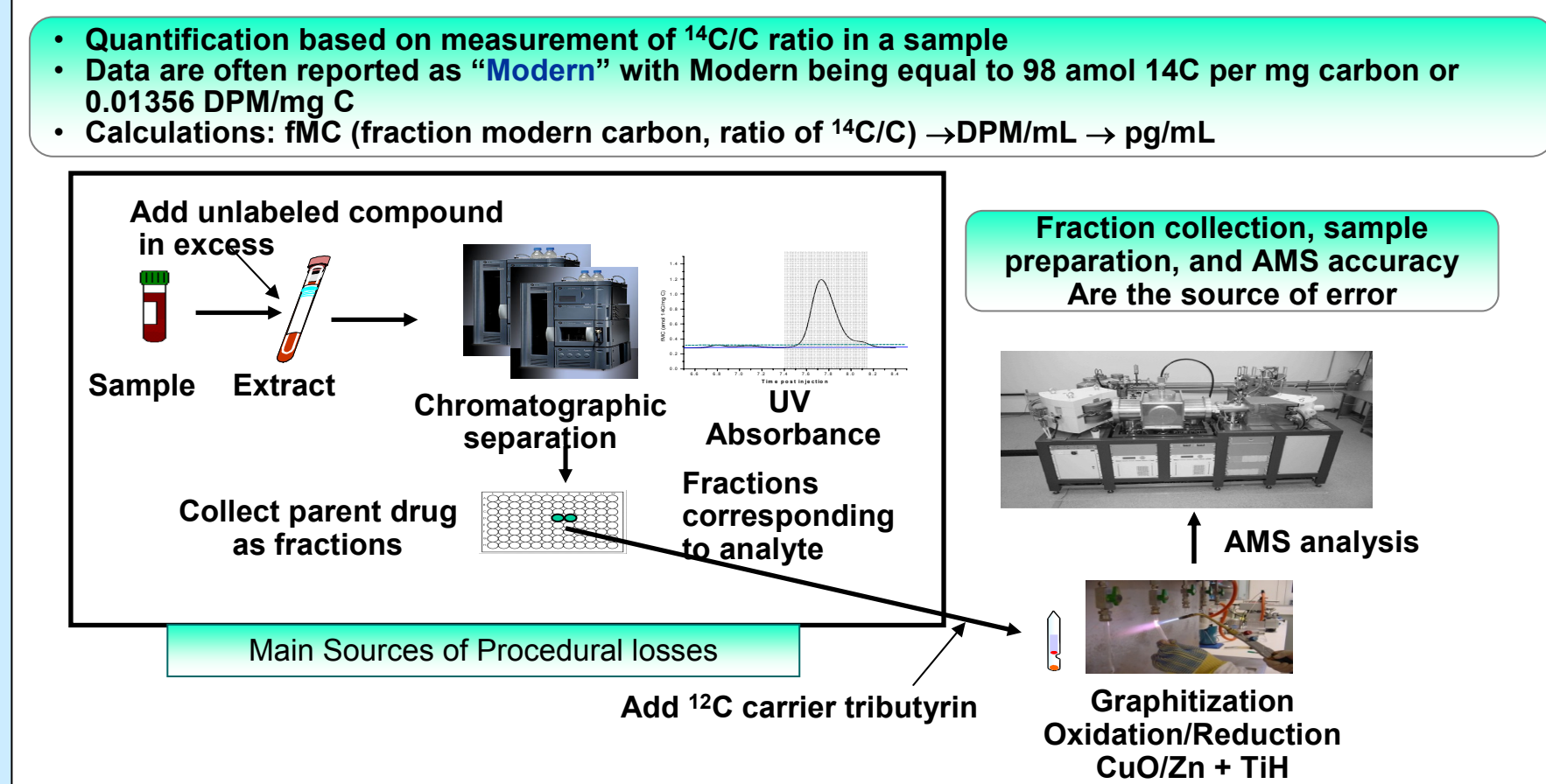
Absolute BA Study Design in Expedient Microdose Approach



Accelerator Mass Spectrometer (AMS)



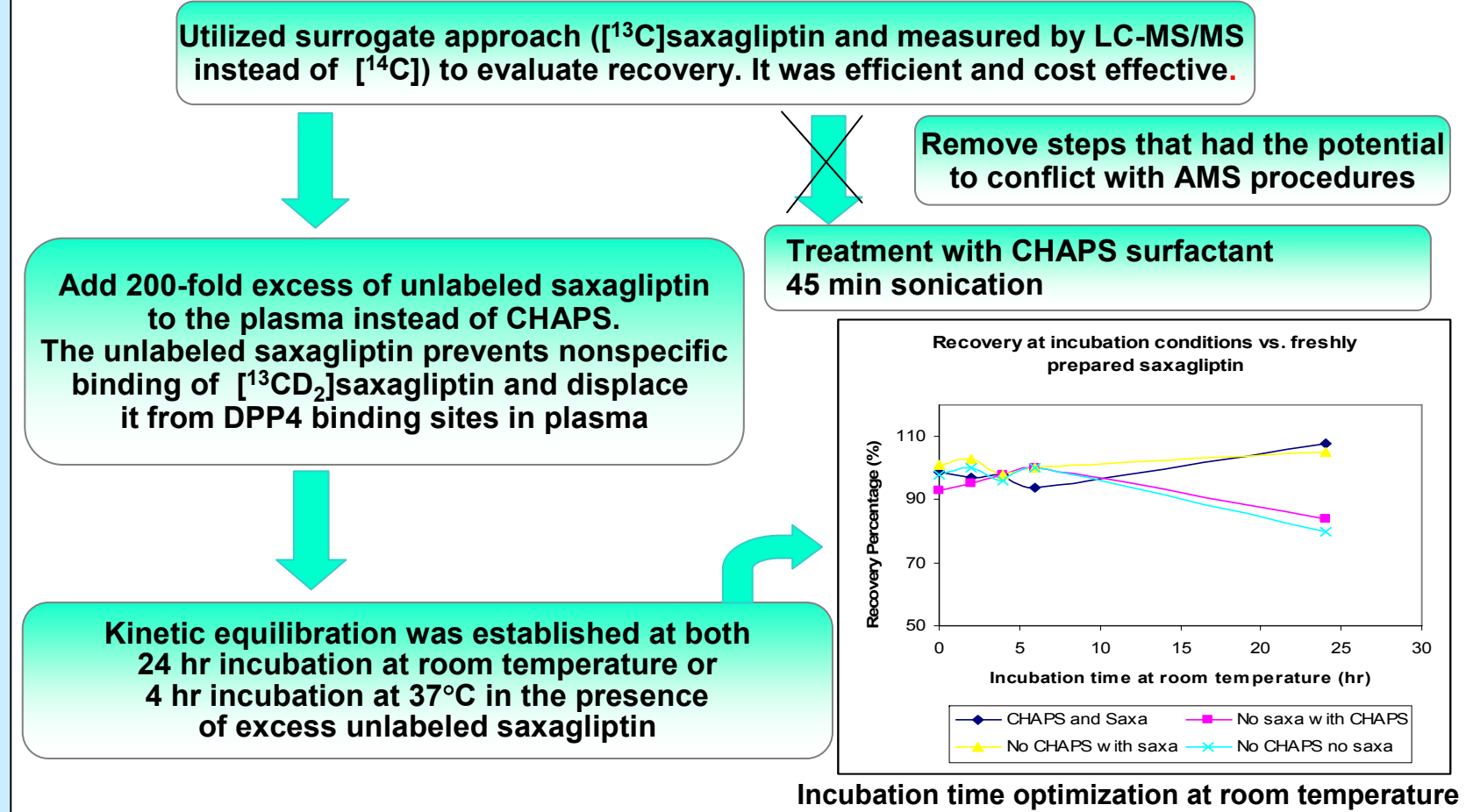
General AMS Process for Absolute BA Measurement



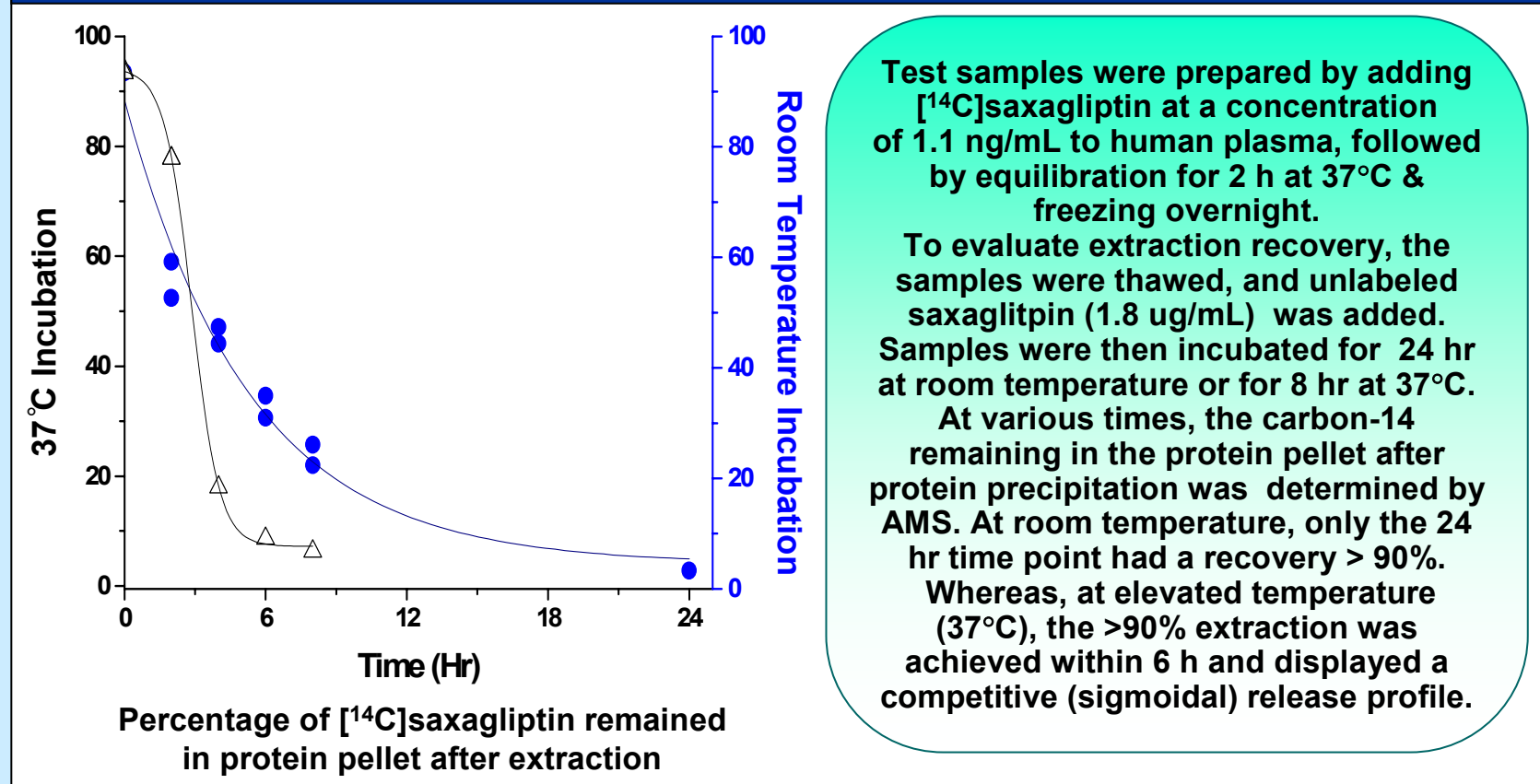
Challenges

- Extraction Recovery**
 - During development of an LC-MS/MS assay for saxagliptin, poor extraction recovery was observed at concentrations below <0.5 ng/mL, presumably due to non-specific binding and/or tight binding of the drug to plasma DPP4.
 - The LC-MS/MS assay utilized "CHAPS" and 45-min heated sonication to overcome extraction losses. However, use of CHAPS may interfere with AMS analysis as it may give high carbon background.
 - In the absence of an internal standard, reliable and high extraction recovery of the [¹⁴C]saxagliptin is critical to achieve the needed sensitivity and reliability in derived concentrations from the AMS analysis.
- Peak Isolation**
 - Chromatographic separation of saxagliptin from its metabolites is critical, since AMS cannot distinguish co-eluting metabolites by mass as it only counts various isotopes of carbon.
 - AMS labs do not have bench-top MS detectors for identification of drug-related components & their retention times (use UV detection).
 - Reference standards for many of the minor metabolites were not available.

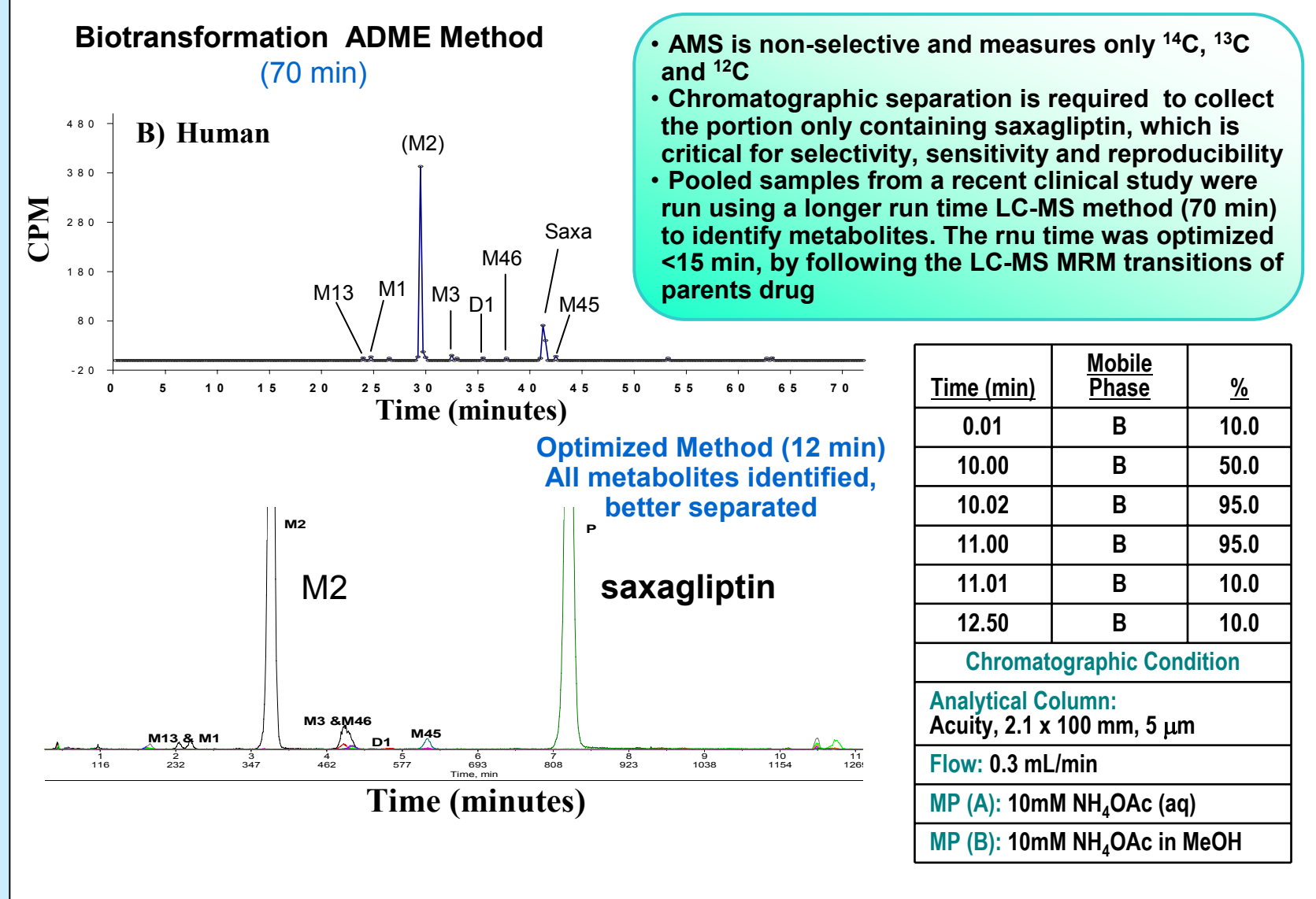
Optimizing Extraction Efficiency



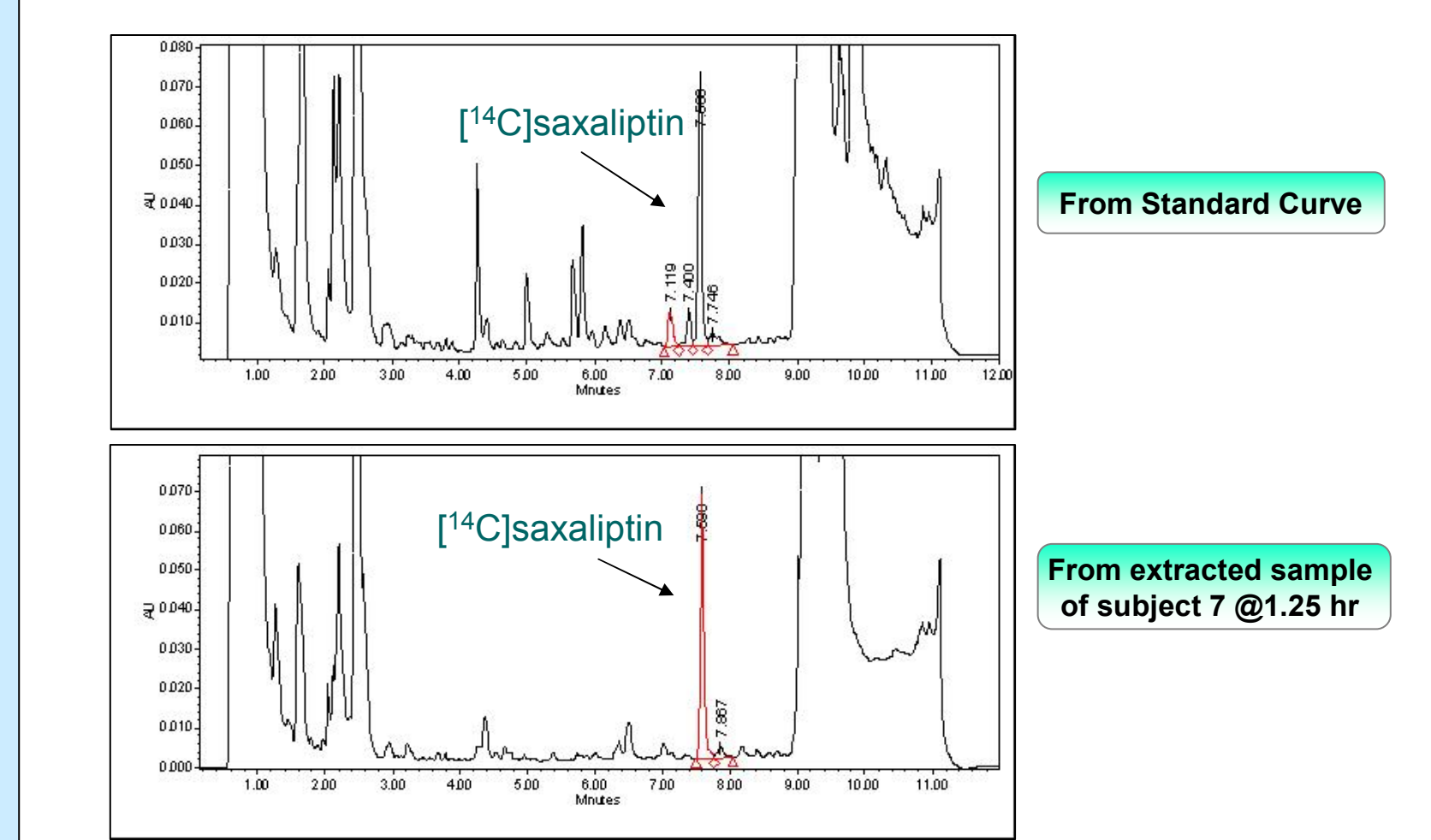
Extraction Recovery of [14C]Saxagliptin from Human Plasma



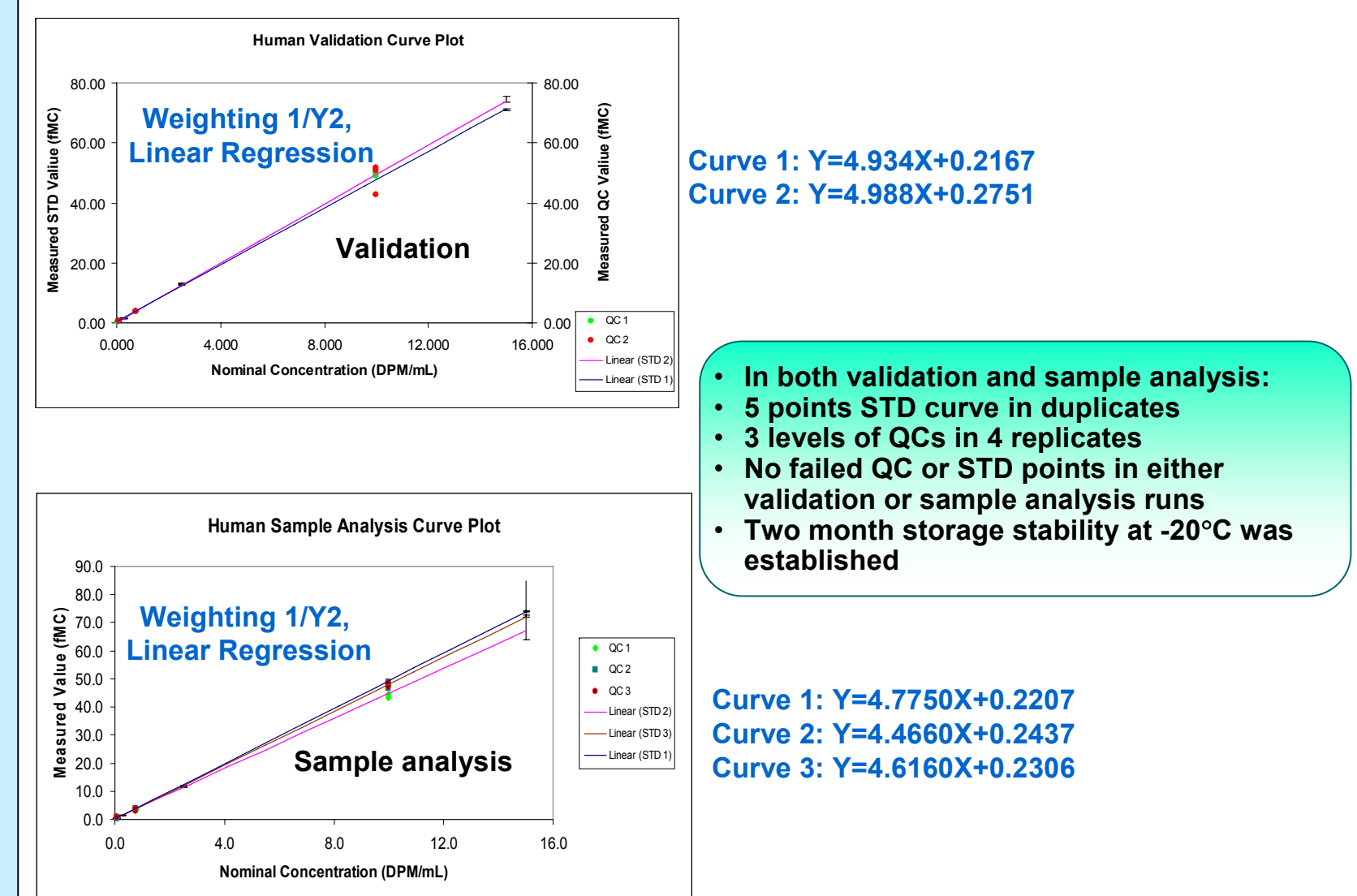
Revised Chromatographic Conditions



Representative UV Chromatograms from Standard and Samples



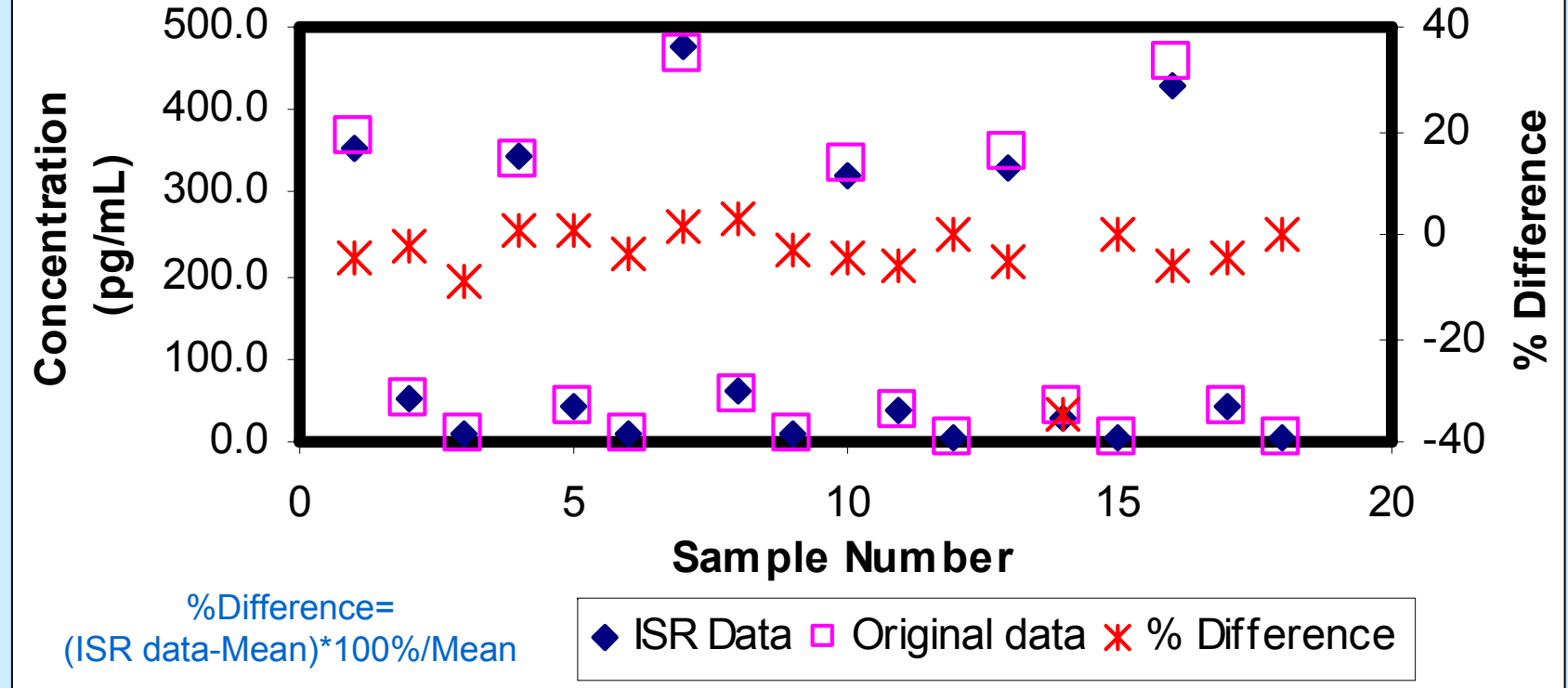
Validation Curve, Sample Analysis Curve and QC Summary



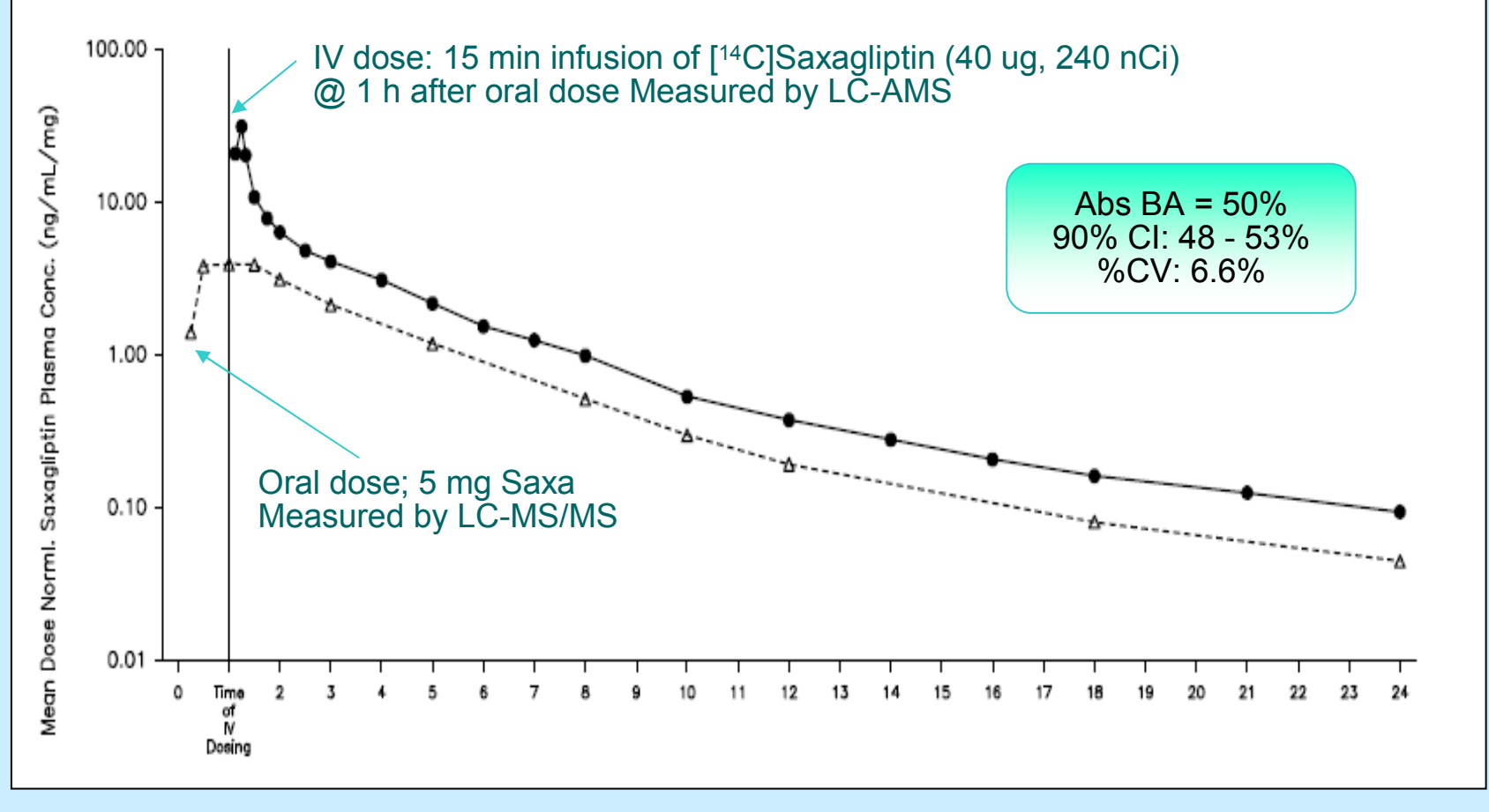
Summary of QC data for the analysis of [¹⁴C]saxagliptin by AMS

Quality Controls	0.075 DPM/mL	0.750 DPM/mL	10.0 DPM/mL
Mean (dpm/mL)	0.0775	0.6859	9.5177
N	4	4	4
%CV	1.4	1.0	4.5
%Accuracy	103.4	91.5	95.2
Mean (dpm/mL)	0.0683	0.7333	10.7034
N	4	4	4
%CV	2.0	4.0	2.1
%Accuracy	91.0	97.8	107.0
Mean (dpm/mL)	0.0726	0.7142	10.3755
N	4	4	4
%CV	2.1	8.8	1.4
%Accuracy	96.8	95.2	103.8

Incurred Sample Reanalysis (ISR) Data



Dose Normalized Mean Plasma Drug Concentration-Time Profile Following Oral and IV Administration to Human Subjects (n=8)



Conclusions

- Extraction recovery was optimized/maximized: Minimized non-specific binding and DPP4 specific binding at low concentrations.
- Metabolite separation from saxagliptin was assured during method development by monitoring components with LC-MS/MS: Minimized background contamination/interference.
- The use of standard curve and QCs may compensate for background ¹⁴C in the system and possibly correct for concentration dependent non-linearities in recovery.
- The raw AMS measurement, ¹⁴C/C ratio, expressed as fMC (fraction Modern Carbon) and known DPM/mL of [¹⁴C]saxagliptin standards, were used to construct a weighted (1/y²) least-squares, linear regression model and used to predict the concentration of unknowns.
- A technique-appropriate validation was conducted that fully demonstrated the accuracy, precision, stability, specificity and recovery of the AMS method across the concentration range of 0.025 to 15.0 DPM/mL (equivalent to 1.91 to 1144 pg/mL).
- The absolute bioavailability of saxagliptin was at ~50%. Pharmacokinetic results indicated good agreement in the terminal phase half-life values between the IV and oral routes demonstrating that the IV radioactive microdose provides a sound approach to determine absolute bioavailability.
- Data from the saxagliptin absolute BA study were included in regulatory filings which results in drug approval.

Acknowledgements

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