Absolute Bioavailability Analysis Using Stable Isotopes: A Liquid Chromatography-Tandem Mass Spectrometry Approach

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PURPOSE

Background:

- Absolute bioavailability studies assess the in vitro levels of a drug administered via oral dose in direct comparison with the same drug administered via intravenous (IV) infusion to determine rates of metabolism.
- Performance of a clinical study of an IV dose requires extensive toxicological testing and intravenous formulations themselves can present some technical challenges.
- Most drugs go to market in an oral formulation only. As a result, obtaining this data can be time-consuming and expensive, with production of the formulation and toxicology data being used only for support of the oral dose.
- For this reason it has been established that using dosages of a microtracer IV formulation at significantly less than the concentration of an oral form does not require the extensive toxicology testing required of a standard pharmacologically relevant drug dosage, yet pharmacokinetic (PK) action of the drug in vivo will present valuable data to characterize the distribution and clearance of the drug in subjects.
- IV administration at a much lower concentration than the oral dose presents analytical challenges when both forms need to be quantified from the same sample.

Analysis:

- Previously, bioavailability studies have used radiolabeled drug forms and accelerator mass spectrometry to gain the sensitivity needed to quantify a microtracer dose.
- Recent advances in the technology of triple quadrupole mass spectrometers have decreased the limits of detection and increased the dynamic range such that LC/MS/MS has become a viable and reliable tool to meet the sensitivity requirements.
- The speed of LC/MS/MS assays and the relatively minimal sample preparation required, compared to accelerator mass spectrometry analyses, have made this tool an asset in the execution of absolute bioavailability studies.
- Selectivity of LC/MS/MS in analysis of each drug form is achieved with distinct masses of the stable isotopically labeled material. Reliability of the data is increased when the chemical nature of the formulations is identical.
- For the work described here, stable isotopically labeled drug material was used for each formulation and quantified distinctly via LC/MS/MS. Inclusion of a third stable isotope internal standard (IS) also increased the robustness of the analysis.

Consideration for LC/MS/MS analysis:

- Challenges exist in producing three distinct isotopic forms with acceptable isotopic selectivity for use in mass spectrometry.
- Mass channel contribution from naturally occurring isotopes becomes a concern when the concentration of the parent and the isotopically labeled co-administered form are significantly different.
- Performing isotopic calculations to determine what labeled forms could be used in the study provides a theoretical estimate of levels of isotopic contribution and helps to determine what are appropriate labeled drug forms for representative analytical ranges and concentrations.
- This LC/MS/MS method was developed to achieve sensitivity requirements for the microtracer analytical range and to determine microtracer and oral dose concentrations from a single sample in a single method.
- The increased sensitivity and dynamic range of triple quadrupole mass spectrometry allowed the oral dose drug to be accurately quantified using a stable isotopically labeled internal standard in samples alongside a third microtracer drug form without impact from detector saturation.

Advances in LC/MS/MS detection limits enable the use of stable isotopically labeled drug forms to accurately quantify bioavailability assays.

RESULTS

Figure 1: Isotopic contribution from single injections in agreement with isotopic calculations in reference paper

- The first two panes from left are microtracer/IS mass transitions, showing no isotopic contribution from injection of a 50000-pg/mL oral dose (upper limit of quantitation, or ULOQ) sample.
- The third and fourth panes from left are oral dose transition/IS of the same sample.

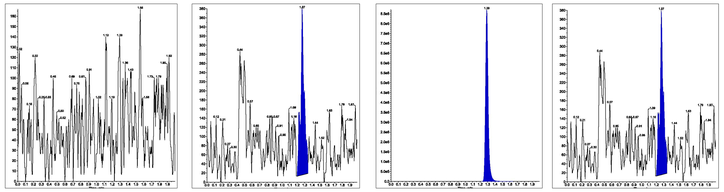


Figure 2: Oral vs. Microtracer LLOQ and ULOQ comparison

- Difference in signal between the oral dose and microtracer can be seen in the chromatograms. However, both analytes were able to be analyzed in tandem with optimized conditions because of the dynamic linear range capabilities of the API 6500 mass spectrometer.

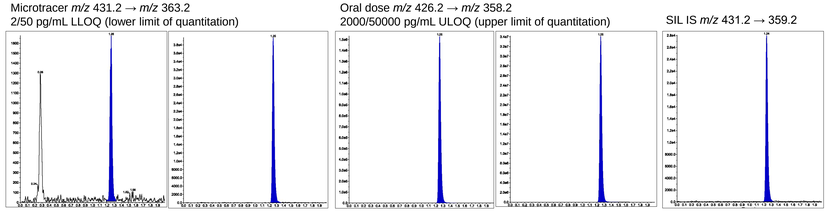
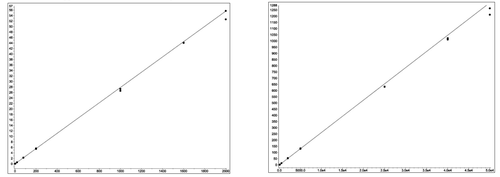


Figure 3: Technical Challenges in Absolute Bioavailability (Dynamic Range Differences)

- Dynamic range capability makes single-injection analysis possible for the microtracer and oral dose forms in direct comparison within each sample.
- Combined curve ranges at 2 to 2000 pg/mL (at left) and 50 to 50000 pg/mL (at right) are illustrated, with two linear curves with minimal detector saturation at 4.0×10^7 response on the oral-dose form using the API 6500 mass spectrometer.



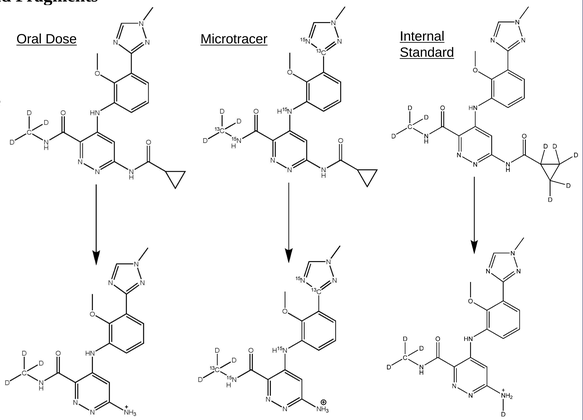
METHOD

- The method was validated across a range of 2 to 2000 pg/mL for the microtracer drug and 50 to 50000 pg/mL for the oral dose form.
- Sample preparation consisted of a buffered liquid-liquid extraction from 100 µL of human plasma; 10 µL of the reconstituted extract was submitted for analysis.
- Separation of background interferences was achieved using gradient elution of 50:950:5:0.5 acetonitrile/water/1 M ammonium formate (aq)/formic acid and 950:50:5:0.5 acetonitrile/water/1 M ammonium formate (aq)/formic acid on a Waters HSS T3 UHPLC column.
- Mass spectrometry analysis used a Sciex API 6500 mass spectrometer and the following mass transitions:

Analyte	Transition monitored
Microtracer [$^{13}C_2$, $^{15}N_3$]-d_3 drug form	*m/z* 431.2 → *m/z* 363.2
Internal standard -d_8 drug form	*m/z* 431.2 → *m/z* 359.2
Oral dose -d_3 drug form	*m/z* 426.2 → *m/z* 358.2

Structures and Fragments

- The same fragmentation pattern was followed to ensure accurate assessment of isotopic mass contribution in each transition monitored.



CONCLUSION

This methodology allows the lowest limit of detection required for the analysis of the IV formulations and the longest dynamic range to streamline analysis and provide high quality data for both oral and intravenous microtracer compound from a single injection method.