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Practical strategies when using a stable isotope labeled microtracer for absolute bioavailability assessment: A case study of a high oral dose clinical candidate GDC-0810

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ABSTRACT

The pH labile metabolite, hydrophobicity, high oral dose and systematic exposure of GDC-0810 posed tremendous challenges to develop a LC–MS method for a stable isotope labeled aBA study. In this study, we explored practical solutions to balance stability and sensitivity and to cope with the impact of high $C_{p.o.}$ to $C_{i.v.}$ ratio on the labeling selection and assay dynamic range. A $[^{13}C_9]$ GDC-0810 was synthesized to minimize the isotopic interference between PO dose, internal standard and I.V. microtracer. A highly sensitive LC–MS assay was validated for quantitation of $[^{13}C_9]$ GDC-0810 from 5 to 1250 pg/mL. The optimized method was applied to a proof of concept cynomolgus monkey aBA study and the bioavailability calculated using microtracer dosing and regular dosing were similar to each other.

1. Introduction

With increasing sensitivity of mass spectrometry, there is a growing interest in pharmaceutical industry to use stable isotope labeled($^{13}C/^{15}N$) microtracer and regular LC–MS for bioanalysis when conducting aBA studies [1–6]. Compared to accelerator mass spectrometry(AMS) quantitation of radiolabeled microtracer, this approach eliminates the lengthy assay development and potential variation caused by chromatographic separation, fraction collection and graphitization in AMS [7]. These advantages are especially useful for compounds with closely eluting or interconvertible metabolites.

When the oral exposure is significantly higher(>1000 fold) than $^{13}C/^{15}N$ labeled microtracer i.v. exposure, Isotope interference from unlabeled compound to microtracer and internal standard will become more severe and impact the labeling strategy. The dynamic range of mass spectrometer detection could also limit the possibility of quantitating oral and i.v. exposure simultaneously. In the previously published four aBA studies using this approach, the ratio of $Cmax_{p.o.}/C_{0i.v}$ was between 5 and 1000 (see Table 1). Most of these studies measured the oral drug and I.V. microtracer simultaneously with one method at a fixed ratio, with the exception of d Vries et al. [5], where up to 10000:1 ratio was evaluated. In reality, the ratio of unlabeled to labeled drug in standards and QCs are not always consistent with the incurred sample. Due to the cap of 100ug total dose for microtracer set by FDA [8], the higher the oral dose is, the more likely such discrepancy will impact quantitation accuracy for the labeled microtracer.

GDC-0810 is a selective estrogen degrader developed for ER(estrogen receptor) positive metastatic breast cancer. The oral dose used for Phase II trial of GDC –0810 was 600 mg and observed $Cmax_{p.o}$ was 11.4 µg/mL. The projected $C0_{i.v}$ for 100 µg intravenous dose is 4 ng/mL, making the ratio of $Cmax_{p.o.}/C_{0i.v.}$ near 3000 at Tmax, 3 times higher than the highest ratio reported previously. The hydrophobic nature of the compound also increased difficulty of eliminating matrix suppression to achieve desired sensitivity. In

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Table 1
Dosing and exposure information of published aBA study using isotope labeled microtracers.

Compound	Oral dose	IV dose	Number of labels	$C_{p.o.}/C_{i.v.}$	Reference
Tofogliflozin	20 mg	0.1 mg	6	109	[3]
Daclatasvir	60 mg	0.1 mg	6	59	[2]
evacetrapib	130 mg	0.175 mg	8	298	[4]
ibrutinib	560 mg	0.1 mg	6	5.29	[5]
beclabuvir	150 mg	0.1 mg	6	1000	[6]
GDC-0810	600 mg	0.1 mg	9	2830(expected)	

this study, multiple factors impacting recovery and matrix effect were investigated to achieve the desired quantitation limit. The method was applied to a cyno study with equivalent PO/IV ratio of human. We assessed the impact of high $C_{p.o.}/C_{i.v}$(Ratio of C_{max} for oral dosing and C_0 of i.v. dosing). on label selection for the microtracer and internal standard before the in life study. A range of unlabeled to labeled compound concentration ratios similar to the projected $C_{p.o.}/C_{i.v}$ were assessed for their impact on quantitation accuracy. Due to high $C_{p.o.}/C_{i.v}$ ratio, oral and I.V. exposure were measured with two different methods. After data analysis additional testing was carried out to ensure quantitation accuracy when facing higher than expected PO/IV ratio.

2. Experimental

2.1. Chemicals and reagents

The standards of GDC-0810 (purity 99.0%), [$^{13}C_9$] GDC-0810 (purity 99.0%), and GDC-0810-d_5 (Internal standard, IS, purity 98.0%) were synthesized in-house. Acetonitrile (ACN, HPLC grade) was purchased from Thermo Fisher Scientific Inc. (Fairlawn, NJ, USA). Water was purchased from JT Baker Chemical Company (Phillipsburg, NJ, USA). Formic acid and Heptane were purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Ammonium acetate was purchased from Fluka (Steinheim, Germany). Citric acid was purchased from Sigma(St. Louis, MO, USA) Dimethyl sulfoxide (DMSO) (all G.R. grade) was purchased from EMD Millipore (Billerica, MA, USA). Ethyl acetate was purchased from Macron (Charlotte, NC, USA). Blank cynomolgus monkey plasma was purchased from Bioreclamation IVT (Baltimore, MD, USA).

2.2. Instrumentation

The analytical system was composed of a Shimadzu Nexera UHPLC system (Shimadzu Corporation, Kyoto, Japan) and a Sciex Qtrap 6500 quadrupole – linear ion trap mass spectrometer (AB Sciex, Foster City, CA, USA). Analytical separation was performed on a Kinetex C18 column (2.1 mm × 50 mm; 2.6 μm) (Phenomenex, Torrance, CA, USA). A prefilter was used to protect the analytical column.

2.3. Liquid chromatography and mass spectrometry conditions

Extracted samples were analyzed using reversed-phase liquid chromatography. The mobile phases were water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B). The gradient is as the following (t(min), %B)): (0, 20%; 1.2, 60%; 2.2, 60%, 2.21, 20%; 3, 20%). The flow rate was 0.6 mL/min and the total run time for one sample was 3 min. The column temperature was kept at 45 °C and the injection volume was 10 μL.

Mass spectrometric detection was carried out on a Qtrap 6500. The ESI source was operated in positive mode. The curtain, nebulizer, and turbo-gas (all nitrogen) pressures were set at 50, 60, and 60 psi, respectively. DP, EP, CE and CXP were set to be 130, 10, 30, and 20 respectively. MRM transitions were set at 456 → 308 for [$^{13}C_9$]GDC-0810 and 454 → 306 for the IS($[M+2]^+$ was monitored to minimize interference, see Section 3.1 for more details). The source temperature was 600 °C and the ion spray needle voltage was 5500 V. Acquisition in multiple reaction monitoring (MRM) mode was performed with dwell time of 50 ms for [$^{13}C_9$] GDC-0810 and IS.

2.4. Preparations of stock solutions, calibration standards and quality control samplesss

Stock solutions of GDC-0810 (10 mg/mL) and [$^{13}C_9$] GDC-0810 (100 μg/mL) were prepared in duplicate by dissolving the accurately weighed standard in DMSO: ACN (50/50, v/v) for preparation of calibration standards and QC(quality control)s, respectively. The internal standard GDC-0810-d_5 working solution (IS_{ws}, 2 ng/mL) was diluted from stock solution (100 μg/mL) with DMSO: ACN (50/50, v/v).

The intermediate working standard solutions were prepared at concentrations of 75,000 ng/mL of GDC-0810 and 25 ng/mL of [$^{13}C_9$] GDC-0810 by dilution of the primary solution (10 mg/mL of GDC-0810, 100 μg/mL of [$^{13}C_9$] GDC-0810) with DMSO: ACN (50/50, v/v). By diluting the intermediate working standard solutions with cynomolgus monkey plasma, the final concentrations of GDC-0810 standard calibration in cynomolgus monkey plasma samples were 15, 30, 75, 210, 600, 1875, 3000 and 3750 ng/mL, and the final concentrations of [$^{13}C_9$] GDC-0810 standard calibration in cynomolgus monkey plasma samples were 5, 10, 25, 70, 200, 625, 1000 and 1250 pg/mL.

Quality control (QC) spiking solutions were prepared at concentrations 150 μg/mL of GDC-0810, and 50 ng/mL of [$^{13}C_9$] GDC-0810 by diluting the primary solution (10 mg/mL of GDC-0810, 100 μg/mL of [$^{13}C_9$] GDC-0810) from a separate reference material weighing with DMSO: ACN (50/50, v/v). Lower limit of quantitation (LLOQ), low, low-medium, medium, high, and dilution QC1 and dilution QC2 samples were prepared at concentrations of 15, 45, 240, 1500, 2880, 7500 and 75,000 ng/mL of GDC-0810 and 5, 15, 80, 500,960, 2500 and 25,000 pg/mL of [$^{13}C_9$] GDC-0810, respectively, by diluting the QC spiking solutions with cynomolgus monkey plasma. Additional QCs with unlabeled to labeled GDC-0810 ratio at 1000:1 and 10,000:1 were prepared at 15 pg/mL and 960 pg/mL level for [$^{13}C_9$]GDC-0810.

After data analysis, retrospective assessment QCs(RAQCs) were made at 50 pg/mL and 70 pg/mL with unlabeled to labeled ratio at 25,000:1 and 50,000:1 respectively to ensure quantitation accuracy of the *in-vivo* sample.

2.5. Sample preparation

Cynomolgus monkey plasma sample were treated by 2 M citric acid to achieve a concentration of 40 mM right after sample collection. A total of 250 μL cynomolgus monkey plasma sample was spiked with 25 μL of IS (2 ng/mL) solution and 225 μL 10 mM Ammonium Acetate. After a thorough vortex mixing for 3 min, the mixture (400 μL) was transferred onto ISOLUTE SLE+ 400 μL(solid supported liquid extraction) plate for extraction. 2 × 0.8 mL of Ethyl Acetate: Heptane (80:20) was then added to each sample on the SLE plate for elution. The eluted extract was then dried down under nitrogen. Samples were reconstituted using 0.3 mL of methyl-*tert*-butyl-ether (Fisher Scientific, Pittsburgh, PA, USA) and dried down under a nitrogen. Samples were then reconstituted again by 100 μL of Acetonitrile: Water: Formic acid (50:50/0.1,v/v/v). Finally, 10 μL of the solution was injected on to the UPLC–MS/MS system.