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ARTICLE

A microdosing framework for absolute bioavailability assessment of poorly soluble drugs: A case study on cold-labeled venetoclax, from chemistry to the clinic

Amr Alaarg[1] | Rajeev Menon[2] | David Rizzo[3] | Yemin Liu[4] | Jeffrey Bien[5] | Tricia Elkinton[6] | Timothy Grieme[7] | Lutz R. Asmus[8] | Ahmed Hamed Salem[2,9]

[1]Drug Product Development, AbbVie Inc., North Chicago, Illinois, USA

[2]Clinical Pharmacology and Pharmacometrics, AbbVie, North Chicago, Illinois, USA

[3]DMPK-BA, AbbVie Inc, North Chicago, Illinois, USA

[4]Development Sciences Analytical R&D, AbbVie Inc., North Chicago, Illinois, USA

[5]Development Sciences Program Management & Sourcing, North Chicago, Illinois, USA

[6]AbbVie Clinical Pharmacology Research Unit, Grayslake, Illinois, USA

[7]Process R&D AbbVie Inc., North Chicago, Illinois, USA

[8]Drug Product Development, AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

[9]Clinical Pharmacy, Ain Shams University, Cairo, Egypt

Correspondence
Ahmed Hamed Salem, Clinical Pharmacology and Pharmacometrics, AbbVie Inc., Dept. R4PK, Bldg. AP31-3, 1 North Waukegan Road, North Chicago, IL 60064, USA.
Email: ahmed.salem@abbvie.com

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Abstract

This work presents an end-to-end approach for assessing the absolute bioavailability of highly hydrophobic, poorly water-soluble compounds that exhibit high nonspecific binding using venetoclax as a model drug. The approach utilizes a stable labeled i.v. microdose and requires fewer resources compared with traditional approaches that use radioactive ^{14}C-labeled compounds. The stable labeled venetoclax and internal standard were synthesized, then an i.v. formulation was developed. In the clinical study, female subjects received a single oral dose of venetoclax 100 mg followed by a 100-μg i.v. dose of cold-labeled ^{13}C-venetoclax at the oral time of maximum concentration (T_{max}). The i.v. microdose was prepared as an extemporaneous, sterile compounded solution on the dosing day by pharmacists at the clinical site. Several measures were taken to ensure the sterility and safety of the i.v. preparation. A sensitive liquid chromatography-tandem mass spectrometry method was developed to allow the detection of plasma levels from the i.v. microdose. Plasma samples were collected through 72 h, and pharmacokinetic parameters were estimated using noncompartmental methods. Postdosing sample analysis demonstrated the consistency of the preparations and allowed the precise calculation of the pharmacokinetic parameters based on the actual injected dose. The absolute bioavailability of venetoclax was estimated at 5.4% under fasting conditions. Venetoclax extraction ratio was estimated to be 0.06 suggesting that the fraction transferred from the enterocytes into the liver is limiting venetoclax bioavailability. The proposed framework can be applied to other highly hydrophobic, poorly water-soluble compounds that exhibit high nonspecific binding to support the understanding of their absorption and disposition mechanisms and guide formulation development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Microdosing is an approach to obtain pharmacokinetic data of a drug and its formulation using a sub-pharmacologic dose.

interpretation of data as well as the writing, review, and approval of the manuscript.

WHAT QUESTION DID THIS STUDY ADDRESS?

Can a stable-label intravenous microdose of highly hydrophobic, poorly water-soluble compound be prepared and safely administered in human subjects to assess the compound's absolute bioavailability? What is the absolute bioavailability of venetoclax?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The proposed framework enabled the determination of the absolute bioavailability of venetoclax and suggests that the fraction transferred from the enterocytes into the liver is limiting venetoclax bioavailability. This finding will support the understanding of venetoclax absorption and disposition mechanisms which are critical for formulation development.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Using venetoclax as a model drug, this work provides a comprehensive framework for assessing the absolute bioavailability of highly hydrophobic, poorly water-soluble compounds that exhibit high nonspecific binding by utilizing a stable labeled i.v. microdose; thus requiring fewer resources when compared with traditional approaches that use radioactive ^{14}C-labeled compounds.

INTRODUCTION

Microdosing is an approach to obtain pharmacokinetic data of a drug and its formulation using a subpharmacologic dose. Such an approach is typically applied early in the drug development before phase I studies (e.g., phase 0, exploratory studies), with inherently safe dosing levels in humans. The pharmacokinetic data obtained from such clinical studies can enable early go/no-go decisions for drug candidates before entering the more expensive clinical drug development programs. Microdosing can also be used later in drug development to estimate absolute bioavailability. This estimation can guide the formulation development activities of non-i.v. (e.g., oral) medications by identifying the characteristics that may present challenges, such as absorption or the first-pass effect.

Absolute bioavailability (F) is the amount of drug from a non-i.v. formulation that reaches the systemic circulation relative to an i.v. dose. Typically, it is determined by a crossover study design comparing an oral product to an i.v. one and using clinically relevant doses or doses that provide an adequate pharmacokinetic profile. However, developing a solution for i.v. administration of poorly water-soluble drugs is challenging. Furthermore, clinical doses of i.v. solution may not be feasible due to safety concerns and solubility challenges.

Intravenous microdose (≤100 μg or 1/100th of the therapeutic dose), labeled with a ^{14}C radiotracer, has been used to determine absolute bioavailability in several studies.[1,2] The labeled dose is typically administered at the time of the peak plasma level (C_{max}) of the nonlabeled oral dose. However, applying such radioactive traces requires extensive safety measures. Such safety measures limit the number of clinical sites where such an i.v. dose is prepared and administered, increasing the study cost. Advances in ultra-sensitive bioanalytical techniques, including accelerated mass spectrometry, enable the analysis of stable labeled (SL), nonradioactive (e.g., ^{13}C) drugs down to subnanogram/ml levels in human plasma.[3,4] Such techniques allow the use of SL drugs instead of radiotracers for a microdosing approach.

The preparation of a nontypical, sterile, i.v. solution of an SL hydrophobic drug remains challenging. The limited number of doses for such relatively small studies, low solubility in aqueous media, and low drug concentration (typically <20 μg/ml) can hinder the preparation of a safe i.v. microdose solution with adequate volume for injections. Drugs with high nonspecific binding hinder accurate delivery of the microdose, which can significantly impact the precise calculation of F.

Venetoclax is a selective B cell lymphoma–2 inhibitor indicated for the treatment of patients with chronic lymphocytic leukemia and acute myelogenous leukemia. Venetoclax is a Biopharmaceutic Classification System (BCS) class i.v. compound (i.e., low solubility and low permeability) with high nonspecific binding and is available as an oral tablet.[5] Venetoclax pharmacokinetics and pharmacodynamics have been well-characterized in healthy volunteers as well as in patients with chronic lymphocytic leukemia, acute myelogenous leukemia, multiple myeloma, or non-Hodgkin lymphoma.[6–12] Venetoclax reaches C_{max} 6–8 h after dosing, and has a terminal half-life of 16 h.[13,14] Venetoclax is predominantly metabolized

by the CYP3A4 enzyme.[15,16] The relationship between venetoclax exposure and clinical responses has been previously described.[17–20] However, venetoclax absolute bioavailability has not been assessed. In this paper, we present an end-to-end case study on using a sterile, i.v. solution of ^{13}C labeled microdose for evaluating the absolute bioavailability of venetoclax, from synthesis to the clinic.

MATERIALS AND METHODS

Synthesis of ^{13}C-venetoclax

The workflow of the development is outlined in Figure 1 and is detailed below. The design of the SL compounds (1 and 2) in Figure 2 for the i.v. microdosing study of venetoclax incorporated the commercially available labeled building blocks. The $^{13}C7$ tert-butyl-4-bromo-2-fluorobenzoate was used for the synthesis of labeled [$^{13}C7$]-venetoclax, which offered sufficient stable labels to distinguish it from the unlabeled venetoclax.[21] The Stable-Label Internal Standard (SLIS) [$^{13}C7D8$]-venetoclax for the bioanalytical assay incorporated an additional eight stable labels utilizing the available commercial boc-D8-piperazine (Figure S1). This SLIS ensured sufficient separation from the labeled parent ^{13}C-venetoclax during the bioanalysis by liquid chromatography-mass spectrometry (LC-MS).

Formulation development of venetoclax microdose solution

The preparation of the venetoclax solution for infusion (Figure 3) started with dissolving venetoclax drug substance (AbbVie Inc.) in polyethylene glycol 400 (PEG-400; Dow Chemical Company) to prepare a 4 mg/ml starting stock solution. Subsequently, the venetoclax-PEG 400 solution was mixed and diluted in a 1:20 ratio into a 10% (w/w) solution of 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD; Wacker) in sterile saline solution (Hospira) to achieve a working stock solution of a target concentration of 0.2 mg/ml. The prepared drug substance solution was further diluted into sterile saline to a final target concentration of 15 μg/ml of ^{13}C-venetoclax post-filtration.

The ^{13}C-venetoclax microdosing aqueous solution for infusion was compounded at the AbbVie Clinical Pharmacology Research Unit (ACPRU) following USP <797> standards for a high aseptic risk, compounded sterile preparation. Under International Organization for Standardization (ISO) class 5 room condition, two sterile 0.2 μm polyethersulfone (PES) filters (Pall) were connected with a sterile connector (BD). The filter/connector set-up was saturated with 10 ml of the 0.2 mg/ml solution followed by flushing with 10 ml of the solution at the intended final concentration of 15 μg/ml. The saturated and flushed filter/connector setup was then used to filter the 15 μg/ml final solution into a sterile empty glass vial (Hospira).

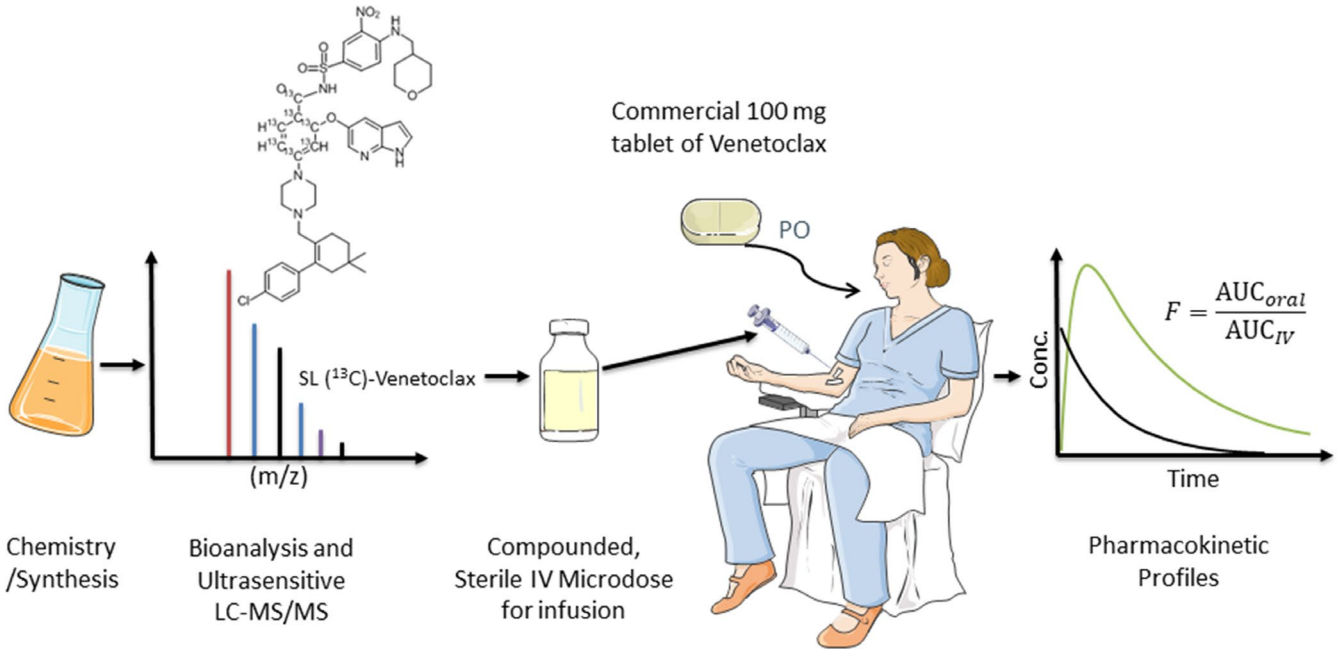
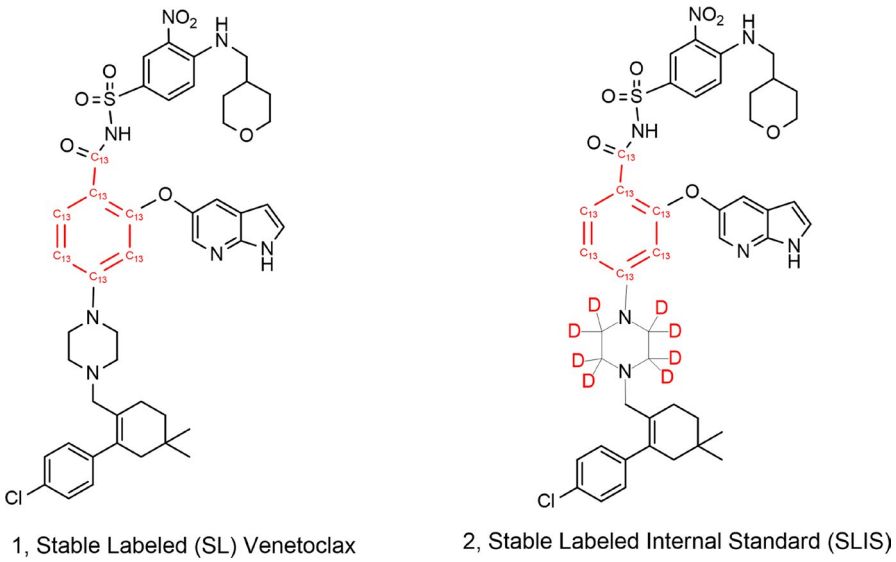


FIGURE 1 The microdosing approach for the assessment of the absolute bioavailability of venetoclax. AUC_{IV}, area under the curve following intravenous drug administration; AUC_{oral}, area under the curve following oral drug administration; F, absolute bioavailability; SL, stable-label; MS, mass spectrometry; LS-MS/MS, liquid chromatography-tandem mass spectrometry

FIGURE 2 Labeled compounds used in the study



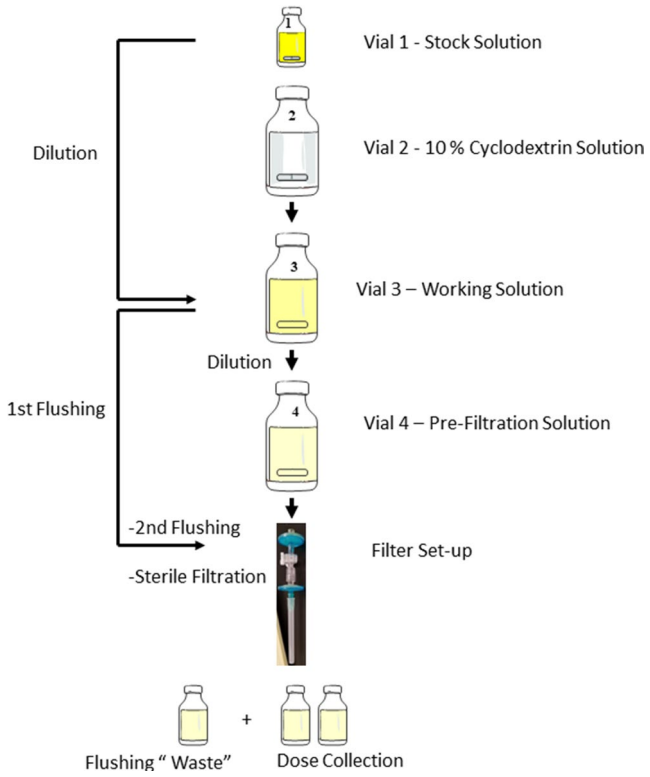


FIGURE 3 The process for the extemporaneous preparation of venetoclax microdosing solution

Microbiological control strategy, sterility, and endotoxin

A risk assessment concerning sterility and endotoxin content was conducted to assess the steps and the conditions of preparing the high-risk compounded sterile preparation of venetoclax, and mitigation strategies were developed to ensure the safety of the venetoclax microdose. This assessment was performed at several layers, including incoming materials, ancillaries, environmental controls, equipment, handling, and the allowable beyond-use time. As an example, dose preparation in an ISO class 5 environment was mandated per USP <797> requirements. The preparations were performed using pre-sterilized ancillaries that are commonly used in a compounding pharmacy, including sterile syringes and sterile empty vials, to minimize the incursion of microbial contamination. The microbiological quality and endotoxin levels of the starting materials were controlled, and the endotoxin contribution of each component to the final dose was calculated.

To ensure the sterility of the dose, the two 0.2 μm filters were tested by filter integrity testing (a bubble point test) after the filtration steps. Both filters were mandated to pass before labeling, dispensing, and transfer of the microdose for administration. All filters used during the clinical study passed the bubble point test.

Drug substance and formulation analytical methods

Drug substance analysis

The potency of the drug substance was determined by quantitative nuclear magnetic resonance (q-NMR) in 1% D2O in DMSO-d6 with a Bruker Avance NMR spectrometer (Billerica, MA), operating at 700 MHz 1H frequency. The level of residual unlabeled drug substance was determined with a Thermo LTQ Orbitrap XL mass spectrometer (Waltham, MA). The bacterial endotoxin level of the drug substance was measured with a BioWhittaker Ex 808 turbidimetric reader (Walkersville, MD).

Drug formulation analysis

The subvisible particles of the formulation solution were measured on a Klotz Particle Counter (Bad Liebenzell, Germany). To determine the venetoclax content in the formulation, a design of experiment (DoE) study was performed to identify the best diluent for extracting the venetoclax from the aqueous formulation. Organic solvent (acetonitrile), surfactant (Tween 80), and sugar (sucrose) were included in the study. After dilution, the API content in the solution was measured on an Agilent 1100 HPLC instrument (Santa Clara, CA, USA) equipped with a Kinetex C8 column (Torrance, CA, USA). The solution pH was measured with a Fisherbrand Accumet AB15 plus pH meter (Pittsburgh, PA).

Clinical study

The clinical study was conducted in accordance with Good Clinical Practice guidelines and ethical principles that have their origin in the Declaration of Helsinki. The protocol was approved by the institutional review board at Vista Health System, Waukegan, IL, and written informed consent was obtained from each patient before any study-related procedures were performed.

Subjects

Enrollment criteria were similar to previous venetoclax studies in healthy volunteers.[22–24] Adult female subjects older than 18 years of age and in general good health were enrolled. The enrollment of female subjects was based on findings in animals, suggesting that male fertility may be compromised by treatment with venetoclax. Subjects must not have used any of the following before study drug administration: any tobacco or nicotine products within 6 months; any investigational product within 42 days; any drug by injection within 30 days; cytochrome P450 (CYP) 3A inhibitors, such as ketoconazole, or CYP3A inducers, such as rifampin and carbamazepine, within 30 days; any over-the-counter prescription medication, vitamin, or herbal supplement within 14 days; warfarin within 7 days; any grapefruit, grapefruit products, Seville oranges, starfruit, or any alcohol within 3 days.

Study design

The study was a single-center, open-label, single-dose study conducted in the United States. Twelve subjects received a single oral dose of venetoclax 100 mg with ~ 240 ml of water. After 4 h, the subjects received a 100-μg i.v. dose of cold-labeled ^{13}C-venetoclax, infused over 15 min using a syringe pump. The i.v. line for the infusions was placed in either upper extremity, ranging from radial area to the ante-cubital region of the arm. The 100 mg dose was selected because it is the highest available strength of the venetoclax formulation.[25] The therapeutic doses cannot be safely administered to healthy volunteers. They can result in prolonged B-lymphocyte reductions, which led to termination of a previous drug interaction study.[23] Venetoclax administration was under fasting conditions (after an approximate 10-h fast and at least 4 h prior to lunch).

Pharmacokinetic sample collection

Plasma concentrations samples for assay of venetoclax and ^{13}C-venetoclax were collected prior to oral dosing, at 1, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h post-oral dose and 5, 10, 15, 20, 25, and 30 min after i.v. dose. To ensure the accuracy of the dose delivered to subjects through the i.v. route, a post-dosing sample was collected for analysis. In detail, the infusion line was not flushed after the end of the infusion. After the completion of the infusion, the i.v. administration set was disconnected, and a post-dosing sample (0.5–1 ml) was collected from the distal end of the infusion line into pre-weighed HPLC vials for analysis. These samples were then assayed for ^{13}C-venetoclax to calculate the actual i.v. dose administered according to the following equation:

$$\text{Actual IV dose} = \text{Concentration of the solution} \times \text{Infusion volume}.$$

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of venetoclax in plasma were estimated using noncompartmental methods in SAS version 9.2 or higher. Pharmacokinetic parameters estimated for the oral dose included the C_{max}, the time to maximum concentration (T_{max}), the terminal-phase elimination rate constant (β), the elimination half-life ($t_{1/2}$), and the area under the curve from time 0 to infinity (AUC_∞). The apparent oral clearance (CL/F) of venetoclax was calculated by dividing the administered dose by AUC_∞. The apparent volume of distribution of venetoclax (Vd_β/F) was calculated by dividing the CL/F by β.

Pharmacokinetic parameters estimated for the i.v. ^{13}C-venetoclax microdose included β, $t_{1/2}$, CL, Vd_β and AUC_∞. The absolute bioavailability of venetoclax was determined as a ratio of dose-normalized AUC_∞ for the oral dose to dose-normalized AUC_∞ for the i.v. dose, as shown in the following equation. The actual i.v. microdose was used in the calculations.

$$F = \frac{AUC_{oral} \times Dose_{IV}}{AUC_{IV} \times Dose_{oral}}.$$

Venetoclax hepatic extraction ratio was also calculated for each subject. Hepatic clearance was assumed to equal the plasma clearance of the i.v. regimen because of the negligible renal clearance.[22] The hepatic extraction ratio was then calculated using plasma clearance, blood to plasma ratio and assuming hepatic blood flow of 90 L/h as follows:

$$\text{Hepatic extraction ratio} = \frac{\text{Plasma clearance (IV Regimen)}}{\text{Blood to plasma ratio} \times \text{Hepatic blood flow}}.$$

Safety and tolerability assessments

Safety was evaluated throughout the study via adverse event monitoring, physical examinations, laboratory tests, vital sign measurements, electrocardiogram assessments, and the number and percentage of subjects reporting treatment-emergent adverse events. Adverse event severity was classified according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 toxicity grade.

Bioanalysis and ultra-sensitive liquid chromatography/tandem mass spectrometry

High-performance liquid chromatography/tandem mass spectrometry analysis of venetoclax and ^{13}C-venetoclax plasma concentrations

Two high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) methods for the measurement of venetoclax and ^{13}C-venetoclax from the same collection sample were developed, validated, and executed internally. The HPLC-MS/MS detection system included a Shimadzu SIL-20ACXR HPLC system (Shimadzu America, Columbia, MD) and an AB Sciex API 5500 triple quadrupole mass spectrometer (Sciex, Framingham, MA) equipped with a TurboIon Spray interface and operated in positive ion mode.

Venetoclax

Venetoclax was isolated from plasma by liquid-liquid extraction by mixing 100 µl of each sample with 50 µl of 50/50 v/v acetonitrile/water containing 550 ng/ml of [$^{13}C_7D_8$]-venetoclax internal standard, adding 600 µl of 50/50 v/v hexanes/ethyl acetate, mixing, and then centrifuging the samples to ensure phase separation. A 400 µl aliquot of the organic supernatant was then transferred to a clean 96-well injection plate, evaporated to dryness, reconstituted in 400 µl of 50/50 v/v acetonitrile/water, and analyzed by HPLC-MS/MS. Chromatography for venetoclax was performed on a dC18 2.1 × 10 mm 3 µm guard column and dC18 2.1 × 50 mm 3 µm analytical column (Waters, Milford, MA) maintained at room temperature. An isocratic elution system was used at a flow rate of 0.4 ml/min, consisting of 55/45/0.1 v/v/v acetonitrile/water/formic acid 88% as the mobile phase and autosampler rinse solution, and 95/5/0.1 v/v/v acetonitrile/water/formic acid 88%) as an autosampler wash solution. Detection was accomplished by selected reaction monitoring (SRM) of the precursor-to-product ion pairs m/z 868.5→321.3 for venetoclax, and m/z 883.4→651.3 for [$^{13}C_7D_8$]-venetoclax internal standard. A total of eight concentration levels were used for venetoclax calibration standards, ranging from 2.14 to 2100 ng/ml.

^{13}C-venetoclax

The ^{13}C-venetoclax was isolated from plasma by liquid-liquid extraction by mixing 200 µl of each sample with 50 µl of 50/50 v/v acetonitrile/water containing 20 ng/ml of [$^{13}C_7D_8$]-venetoclax internal standard, adding 800 µl of 50/50 v/v hexanes/ethyl acetate, mixing, and then centrifuging the samples to ensure phase separation. A 600 µl aliquot of the organic supernatant was then transferred to a clean 96-well injection plate, evaporated to dryness, reconstituted in 100 µl of 50/50 v/v acetonitrile/water, and analyzed by HPLC-MS/MS Chromatography for ^{13}C-venetoclax was performed on a Halo C8 2.1 × 30 mm 2.7 µm (MAC-MOD Analytical, Chadds Ford, PA) maintained at 40°C with a gradient elution system consisting of mobile phase A (10/90/0.1 v/v/v acetonitrile/water/formic acid 98%) and mobile phase B (100/0.1 v/v acetonitrile/formic acid 98%) at a flow rate of 0.5 ml/min during elution. Additionally, dimethyl sulfoxide was used as mobile phase C for a post-elution column wash, and 30/30/30/10 v/v/v/v acetonitrile/methanol/2-propanol/tetrahydrofuran was used as an autosampler wash solution. Mobile phase A was also used as an autosampler rinse solution. Detection was accomplished by SRM of the precursor-to-product ion pairs m/z 875.3→643.4 for ^{13}C-venetoclax, and m/z 883.4→651.3 for [$^{13}C_7D_8$]-venetoclax internal standard. A total of eight concentrations levels were used for ^{13}C-venetoclax calibration standards, ranging from 0.0300 to 15.0 ng/ml.

Quantitation

AB Sciex Analyst version 1.6 software (Sciex) was used to collect and process the data. All samples within a run used the same integration parameters. A peak area ratio versus the concentration of the standards and a weighting of $1/x^2$ (where x is the concentration of a given standard) were used for curve fitting. The regression equation for the calibration curve was then used to back-calculate the measured concentrations. For each standard and quality control (QC), the results were compared to the theoretical concentrations to obtain the accuracy of each level measured. Results from the QC samples were used to verify accuracy and precision of the analytical results for the study samples. Samples with concentrations above the quantitation limit (AQL) for any given run were diluted and assayed with a set of QC samples with the same dilution factor. All reported results were generated within the quantitation limits established by the run calibration curves.

RESULTS

Drug analysis

The potency of the drug substance was 98.9% at the clinical release, and it contained less than 0.1% of residual unlabeled material. The bacterial endotoxin content in this drug substance was determined as 10 EU/mg. This drug substance was stable for a year when stored in a chamber at 25°C with 60% relative humidity.

A mixture of 40% organic solvent and 60% aqueous solution was found the most effective for extracting the venetoclax from the aqueous formulation. The linearity and accuracy of the analytical method using this diluent were demonstrated by the 99–100% recovery of the formulations that cover the range of 2 to 25 μg/ml. The concentration in the filtered dosing vials was reproducible and ranged between 14 and 15 μg/ml after preparation and serial dilution by three different pharmacists. Drug concentration analysis of the venetoclax microdose demonstrated a stable solution in a vial and during the administration with potential adsorption on infusion lines during administration. The analysis of particulate matter for the venetoclax microdose formulation for injection indicated low subvisible particle levels, meeting the requirement of USP <788> Particulate Matter in Injection for both particle greater than 10 μm and greater than 25 μm.

For the analysis in plasma, the lower and upper limits of quantification (LLOQ and ULOQ) for venetoclax were 2.14 to 2100 ng/ml, respectively. A 10-fold dilution was previously validated to accommodate any samples above the upper limit of these ranges. The $[^{13}C_7D_8]$-venetoclax internal standard did not affect the accuracy or precision of the assay, with the QC samples having an intra-run percent coefficient of variation (%CV) less than or equal to 7.1% and an intra-run percent bias of −2.5 to 2.4% (n = 6 replicates of 3 QC levels). As part of the qualification, matrix effects, including 5% hemolyzed and lipemic matrices, were validated and found to have no impact on the assay. Additionally, interference from ^{13}C-venetoclax was also evaluated and found to not interfere with detection and quantitation.

The LLOQ and ULOQ evaluations for ^{13}C-venetoclax were 0.0301 to 15.1 ng/ml, respectively. A 10-fold dilution was validated to accommodate any samples above the upper limit of these ranges. The assay demonstrated the accuracy and precision needed, with the QC samples having an inter-run %CV less than or equal to 9.7% and an inter-run percent bias of 1.3–6.7% (n = 18 replicates of 3 QC levels) over three analytical runs. Both compounds were stable in human plasma during the time required for storage and analysis of the samples. Selected samples were subjected to re-analysis for both analytes to assess the incurred sample reproducibility. For venetoclax, 29/30 of the repeats were within ±20% of the original results, and for ^{13}C-venetoclax 30/30 of the repeats were within ±20% of the original results.

Clinical pharmacokinetics

Twelve healthy female subjects completed the study. The mean (SD) age of subjects was 38 (11) years and the mean (SD) weight was 72 (5) kg. Based on the post-dosing drug solution sample analysis, the actual microdose ranged between 85 and 130 μg and was used in calculations. The mean (± SD) plasma concentration-time profiles and pharmacokinetic parameters for venetoclax oral and i.v. administration are shown in Figure 4 and Table 1, respectively. Median T_{max} after oral administration was ~ 4 h whereas the half-life after both oral and i.v. administration was ~ 14 h. The absolute bioavailability for venetoclax was estimated at 5.4% under fasting conditions. Venetoclax hepatic extraction ratio was estimated as 0.06.

Safety and tolerability

The regimens tested were generally well-tolerated by subjects. There were no deaths or severe adverse events reported during the study. The adverse events reported were all mild with the exception of a single adverse event categorized as moderate. One subject discontinued from the study due to an occurrence of an adverse event of

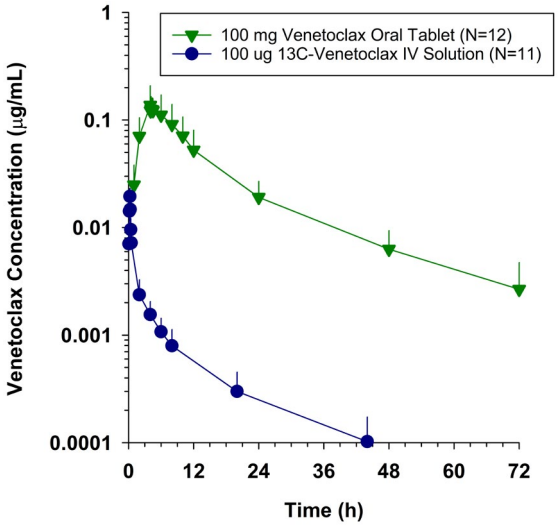


FIGURE 4 Log-linear concentration-time profiles of venetoclax and ^{13}C-venetoclax

TABLE 1 Venetoclax pharmacokinetic parameters

Pharmacokinetic parameters (units)	100 mg venetoclax oral tablet ($N = 12$)	100 µg ^{13}C-venetoclax solution for IV infusion ($N = 11$)
T_{max} (h)	4.2 (4.0–6.0)	0.25 (0.25–0.25)
C_{max} (µg/ml)	0.149 (47%)	0.019 (19%)
AUC_t (µg·h/ml)	1.85 (45%)	0.034 (37%)
AUC_{inf} (µg·h/ml)	1.93 (45%)	0.036 (39%)
$t_{1/2}$ (h)	13.6 (5.1)	14.4 (5.5)
C_{max}/dose (ng/ml)/mg	1.49 (47%)	176 (16%)
AUC_t/dose (ng·h/ml)/mg	18.5 (47%)	310 (36%)
AUC_{inf}/dose (ng·h/ml)/mg	19.3 (45%)	324 (37%)
Absolute BA	5.4% (27%)	
Hepatic extraction ratio	0.06 (38%)	

T_{max} presented as median (range), $t_{1/2}$ presented as harmonic mean (pseudo %CV), all other parameters are presented as mean (%CV).

Abbreviations: AUC, area under the curve; C_{max}, maximum plasma concentration; %CV, percent coefficient of variation; $t_{1/2}$, elimination half-life; T_{max}, time to maximum concentration.

moderate urinary tract infection. No clinically significant vital signs or laboratory measurements were observed during the study.

DISCUSSION

Determining the absolute bioavailability, specifically for poorly soluble drugs, can guide formulation development activities. Such data along with the elimination properties can help identify if poor oral bioavailability is due to absorption or first-pass effect. In this paper, we present an end-to-end approach for assessing the absolute bioavailability of highly hydrophobic, poorly water-soluble compounds that exhibit high nonspecific binding using venetoclax as a model drug. The approach utilizes an SL i.v. microdose and requires fewer resources compared with traditional approaches that use radioactive ^{14}C-labeled compounds.

Developing a sterile, i.v. microdose of poorly soluble drugs is challenging. Due to their hydrophobicity and poor aqueous solubility, these compounds may not be easily compoundable or stable in aqueous matrices, which are suitable for an i.v. dosing. The premise of the formulation process development we implemented was minimizing the time of compounding required for the sterile preparation to mitigate microbiological growth, while assuring sufficient stability. The use of PEG-400 accelerated the dissolution of the poorly soluble venetoclax to less than 15 min and ensured fast and efficient compounding. Serial dilution steps in HP-β-CD in saline ensured the stability of dissolved venetoclax and minimized the drug precipitation upon serial dilution.

Sterile filtration is the only method to assure the sterility and safety of the microdoses for heat-labile drugs that cannot withstand terminal sterilization. However, for hydrophobic drugs, such as venetoclax, adsorptive drug losses may occur on the sterile filters during formulation preparation, and on glass or plastic container surfaces during analytical sample preparation. Such adsorptive losses may impact the estimation of dose accuracy and presented analytical challenges for drug recovery experiments.

Adsorptive losses (1–2 µg/ml) of venetoclax were observed on the infusion line during the formulation and process development. Because the absolute bioavailability calculation is dependent on the exact i.v. dose infused in each patient and with the low concentration for the microdose, any minor variability in the exact dose can impact the precise estimation of the absolute bioavailability. To that end, a flushing process of the PES filter with a concentrated solution and final solution of venetoclax was established to ensure the saturation of the sterile filters before the final step. We also conducted post-dosing drug solution sample analysis throughout the study to estimate the actual dose each subjects received. The actual doses were used directly in the calculation of the absolute bioavailability.

The absolute bioavailability of venetoclax was estimated to be 5.4%. Of note, venetoclax is recommended to be given with food.[26] However, in this study, venetoclax was given under fasting conditions to evaluate the true absolute bioavailability of the drug product in the absence of the solubilizing effect of food. Low-fat and high-fat meals

were found to increase venetoclax bioavailability by 3.4 and 5.1-fold, respectively.[27,28] These results indicate that the effective absolute bioavailability in clinical practice will range between 18% and 28%.

Venetoclax hepatic extraction ratio was estimated at 0.06. This indicates that venetoclax is a low extraction ratio drug with only 6% metabolized by the liver in a single pass. This estimate along with an absorption fraction of almost 1 under fed conditions suggests that the fraction transferred from the enterocytes into the liver is limiting venetoclax bioavailability.[22] Consistently, the i.v. regimen was associated with lower interindividual variability in pharmacokinetic parameters than the oral regimen given under fasting conditions.

One of the challenges of cold-labeled microdosing is the need to develop an ultrasensitive assay that can detect the levels of the ^{13}C drug in the plasma from the i.v. dose. Due to the high sensitivity needed for the detection of ^{13}C-venetoclax at the predicted clinical concentrations, the venetoclax HPLC-MS/MS method had to be redeveloped for ^{13}C-venetoclax. High levels of analyte carryover (~ 200% peak area of the LLOQ) were initially observed, coming from the injection needle, the HPLC system, as well as the analytical column. A washing step was implemented in the autosampler to reduce any analyte carryover coming from the injection. The column was changed from a dC18 to a C8 stationary phase to reduce the analyte retention, and rigorous column washes were added to remove any residual venetoclax between injections. The final HPLC-MS/MS method for ^{13}C-venetoclax had analyte carryover reduced to ~ 50% of the LLOQ peak area, and was monitored throughout sample analysis for any potential impact on quantitation. This new method was fully validated and met all acceptance criteria from the regulatory guidance.

To know how sensitive the assay needs to be, available physicochemical properties and clinical data need to be considered to predict the absolute bioavailability. For example, in this study, the previously observed effects of food, P-gp, BCRP, and OATP inhibition on venetoclax exposure were used to predict the absolute bioavailability and determine the needed sensitivity of the assay.[29–33] For other drugs, where such information could be lacking, the worst-case scenario for absolute bioavailability can be assumed while developing the assay.

Many clinical researchers refrain from conducting microdosing trials because of concerns about the pharmacokinetic nonlinearity when extrapolating the results from the microdosing to the clinical doses. Venetoclax pharmacokinetics is linear between 150 and 800 mg dose.[34] Such concern is only valid when the i.v. microdose is administered in a separate period from the oral dose. In such a scenario, the pharmacokinetic linearity between the i.v. microdose and the oral dose is required to ensure unbiased estimates of the absolute bioavailability. In this study, however, subjects received the i.v. microdose 4 h after the oral dose. The 4-h time point was selected because it is the reported time of maximum concentration after oral administration under fasting conditions.[34] This design eliminates the nonlinearity concerns because the microdose and the oral dose will be exposed to the enzyme and transporter sites at the same time and, hence, they will both be handled similarly.

CONCLUSIONS

The use of the cold-label microdosing approach instead of the traditional radioactive label allowed for dosing in a standard pharmacy equipped to compound sterile doses and did not require a facility equipped to handle radioactive isotopes. The high sensitivity and selectivity of the developed LC-MS/MS method allowed the quantitation of very low levels of ^{13}C-venetoclax in human plasma following administration of an i.v. microdose, and its differentiation from the orally administered, unlabeled venetoclax. Post-dosing sample analysis demonstrated the consistency of the preparations and supported the precise calculation of the pharmacokinetic parameters based on the actual injected dose. Assessing the venetoclax absolute bioavailability will support the understanding of venetoclax absorption and disposition mechanisms, which are critical for any formulation development. The proposed approach can be adopted for other hydrophobic and poorly soluble compounds that exhibit high nonspecific binding.

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CONFLICTS OF INTEREST

R.M., D.R., Y.L., J.B., T.E., T.G., L.R.A., and A.H.S. are AbbVie employees and may hold stock. A.A. was an AbbVie employee at the time this work was conducted and may hold stock.

AUTHOR CONTRIBUTIONS

A.A. and A.H.S. wrote the manuscript. A.A., A.H.S., R.M., L.R.A., Y.L., D.R., J.B., and T.G. designed the research. A.A., D.R., T.E., T.G., and Y.L. performed the research. A.A., R.M., D.R., Y.L., J.B., T.E., T.G., L.R.A., and A.H.S. analyzed the data.

DATA AVAILABILITY STATEMENT

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis

data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications.

This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

ORCID

Ahmed Hamed Salem https://orcid.org/0000-0002-9261-1583

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of the article at the publisher's website.

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