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Comparative Analysis of Ampicillin Plasma and Dried Blood Spot Pharmacokinetics in Neonates

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Abstract

Background—Dried blood spot (DBS) is a practical sampling strategy for pharmacokinetic studies in neonates. The utility of DBS to determine the population pharmacokinetics (pop-PK) of ampicillin, as well as accuracy versus plasma samples, was evaluated.

Methods—An open-label, multicenter, opportunistic, prospective study was conducted in neonates. Ampicillin concentrations from plasma and DBS (CONC_{Plasma} and CONC_{DBS}) were measured by liquid chromatographic tandem mass spectrometry and analyzed using pop-PK and statistical (including transformation) approaches.

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Results—A total of 29 paired plasma and DBS samples from 18 neonates were analyzed. The median (range) gestational age and postnatal age were 37 (27–41) weeks and 8 (1–26) days, respectively. The geometric mean of CONC_{DBS} to $\text{CONC}_{\text{Plasma}}$ ratio was 0.56. Correlation analysis demonstrated strong association between $\text{CONC}_{\text{Plasma}}$ and CONC_{DBS} ($r^2=0.902$, ANOVA $p<0.001$). Using linear regression transformation, the estimated $\text{CONC}_{\text{Plasma}}$ ($\text{eCONC}_{\text{Plasma}}$) was derived using $(\text{CONC}_{\text{DBS}} - 3.223) / 0.51$. The median bias and geometric mean ratio improved to -11% and 0.88 (Wilcoxon signed-rank test, $p<0.001$), respectively, when comparing $\text{eCONC}_{\text{Plasma}}$ to $\text{CONC}_{\text{Plasma}}$. Furthermore, using pop-PK modeling, the median bias (interquartile range) for clearance and individual predicted concentrations improved to 8% (-11 to 50) and -8% (-34 to 11), respectively, when $\text{eCONC}_{\text{Plasma}}$ was used.

Conclusions—After transformation, DBS sampling accurately predicted ampicillin exposure in neonates.

Keywords

dried blood spot; neonates; ampicillin; pharmacokinetic modeling

Introduction

One of the most inherent challenges of pediatric drug studies encompasses the ethics and logistics of blood sampling, in terms of number and volume.¹ Opportunistic study designs capitalize on standard-of-care procedures to minimize the risk to enhance parental consent and curtail research-related costs. These designs have been successfully employed to enhance the dosing of antimicrobial agents in pediatrics, including neonates.^{2–6}

Microsampling is a cutting-edge technique that fosters an ethical approach to pediatric research, especially in neonates where the ability to ascertain a complete pharmacokinetics (PK) profile of the drug is constrained by blood volume and sampling frequency. Dried blood spot (DBS) is a microsampling method that measures concentrations in whole blood and utilizes an ultra-low volume to evaluate the PK of drugs.⁷ As a minimally invasive alternative to venipuncture, the drops of whole blood can be easily obtained from a finger- or heel-stick with minimal requirement for training, therefore facilitating serial sampling for PK studies.⁸ The DBS sampling strategy also eliminates the need for centrifugation or freezing of samples, thereby making the requirements for storage and shipment straightforward and less expensive.^{8,9} Furthermore, the Centers for Disease Control and Prevention reports that shipping DBS samples does not represent an occupational risk of exposure.¹⁰ The practicality and convenience of DBS, as compared with traditional plasma sampling, makes this approach useful for research purposes and has been evaluated in neonates.^{11–13} We designed this study testing the utility and accuracy of DBS versus plasma for determining the population PK (pop-PK) of ampicillin with the ultimate goal of expanding the literature on DBS sampling to potentially enhance its utility in the near future.

Ampicillin is one of the most commonly administered medications in the neonatal intensive care unit (NICU) for early-onset sepsis and bacterial meningitis, so ampicillin was used as a probe drug to evaluate the utility of DBS sampling. Using an opportunistic study design with population-based PK modeling, our study objectives were to 1) characterize the *in vivo*

relationship between $\text{CONC}_{\text{Plasma}}$ and CONC_{DBS} , 2) develop and evaluate a transformation equation that accurately calculates estimated $\text{CONC}_{\text{Plasma}}$ ($\text{eCONC}_{\text{Plasma}}$) using CONC_{DBS} , and 3) evaluate the accuracy of the DBS sampling strategy using pop-PK.

Materials and Methods

Study Design

This prospective, multicenter (N=7) trial was conducted under the PK of Understudied Drugs Administered to Children per Standard of Care (POPS III; protocol NICHD-HHSN27500027) by the Pediatric Trials Network (PTN). The data presented here were compiled from neonates receiving ampicillin. The protocol was reviewed and approved by the institutional review board of each participating institution.

Details of our methodology have been previously published.⁵ Briefly, participating sites were encouraged to collect biological samples opportunistically; therefore, laboratory (including PK and serum creatinine [SCR]) samples were obtained at the time of clinical laboratory evaluations per standard of care. The plasma and DBS PK samples were obtained in pairs. For plasma samples, at least 200 μL (up to $\sim 2000 \mu\text{L}$) of whole blood was collected in an ethylenediaminetetraacetic acid (EDTA)-containing tube and separated by centrifugation at 2000 g for 10 minutes at 4°C . For DBS samples, blood was aliquoted from EDTA prior to centrifugation and 30 μL was spotted on FTA[®] DMPK-C white cards from GE Healthcare Bio-Sciences (Pittsburgh, PA, USA). Both plasma and DBS samples were stored frozen at -70°C and shipped on dry ice until ampicillin concentrations were determined at the central laboratory. The imputation approach for SCR matched the closest record based on date and time.⁵

Bioanalytical Assay

Validated bio-analytical high-performance liquid chromatography with tandem mass spectrometric (HPLC-MS/MS) assays were used to measure the $\text{CONC}_{\text{Plasma}}$ and CONC_{DBS} at the PTN central laboratories OpAns Laboratory (Durham, NC, USA) and Frontage Lab (Exton, PA, USA), respectively. The validated range and limit of quantification of plasma method have been previously published.⁵ For CONC_{DBS} , ampicillin was extracted by protein precipitation from DBS samples using 0.5% formic acid in purified water. Reversed-phase HPLC separation was achieved with a hyperclone BDS column ($150 \times 2.0 \text{ mm}$, 5 micron). MS/MS detection was set at mass transitions of m/z $350.5 \rightarrow 160.1$ for ampicillin in Turbo Ion spray (TIS) positive mode. The validation range for the assay was 0.05–50.0 $\mu\text{g/mL}$. Quality control samples included the following nominal concentrations: 0.15, 5.0, 37.5, and 150 $\mu\text{g/mL}$. The lower limit of quantification (LLOQ) was 0.05 $\mu\text{g/mL}$. Accuracy and precision was assessed using five determinations at theoretical levels and were within the Food and Drug Administration bioanalytical assay validation criteria (e.g., $\pm 15\%$). All sample runs for the study met acceptance criteria.

DBS Analysis and Transformation Methods

Correlation analysis was performed to determine the association between $\text{CONC}_{\text{Plasma}}$ and CONC_{DBS} . Different transformation methods (including ratio, linear regression, and log-

linear regression) were used to derive a formula to calculate $eCONC_{Plasma}$ using $CONC_{DBS}$. The Bland-Altman plot of the ratio of $CONC_{DBS}$ (and $eCONC_{Plasma}$) to $CONC_{Plasma}$ was computed and plotted against mean combined concentrations, together with the 95% confidence interval of the mean ratio.¹⁴ Except for the PK modeling, all analyses were performed using SPSS PASW 22 (SPSS Inc., Chicago, IL, USA).

Population PK Analysis

Using measured $CONC_{Plasma}$ from a small subset of subjects evaluated in a previous study,⁵ data were fitted to a 1-compartment model (ADVAN1 TRANS2) with the First Order Conditional Estimation method with ETA-EPS interaction (FOCE-I) and Bayesian post-hoc estimation in NONMEM 7.2 (Icon, Dublin, Ireland).⁵ To explore differences in population PK parameter estimates with plasma, DBS, and estimated plasma concentrations, we ran the same model three times using each concentration matrix. The bias in concentrations and pop-PK parameter estimates (Y) were assessed by calculating the median percentage prediction error (i.e., $100\% \times [Y_{DBS} \text{ (or } ePlasma) - Y_{Plasma}] / Y_{Plasma}$).

Run management was performed using Pirana (version 2.8.1). Data manipulation and visualization was performed using software R (version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria) and RStudio (version 0.97.551, RStudio, Boston, MA, USA), with the packages lattice, Xpose, and ggplot2 used for the latter. SPSS PASW 22 (SPSS Inc., Chicago, IL, USA) was also used for data visualization and descriptive statistics of patient demographic and lab data.

Results

A total of 29 $CONC_{DBS}$ were sampled at the same time as $CONC_{Plasma}$ from 18 subjects, except for one $CONC_{DBS}$ that was obtained 19 minutes before $CONC_{Plasma}$. The demographic characteristics are summarized in Table 1.

DBS Analysis and Transformation Methods

Using the 29 paired samples, a strong correlation between $CONC_{Plasma}$ and $CONC_{DBS}$ was observed (Spearman's $\rho = 0.97$, $p < 0.001$, Figure 1A). The slopes of the 90% fit loess (with Epanechnikov kernel smoothing) trend lines for the $CONC_{Plasma}$ and $CONC_{DBS}$ were similar, especially at 6-hour time-points. Different transformation methods were evaluated to derive a formula to calculate $eCONC_{Plasma}$ using $CONC_{DBS}$ data (Table 2). Using the simple linear regression method, $CONC_{Plasma}$ was a significant predictor of $CONC_{DBS}$ (where $[CONC_{DBS}] = 3.223 + 0.51 * [CONC_{Plasma}]$; $r^2 = 0.902$, ANOVA $p < 0.001$; Figure 1B). Approximately 90% of the variability in $CONC_{DBS}$ was explained by $CONC_{Plasma}$. In addition, the mean $eCONC_{Plasma}$ was similar to $CONC_{Plasma}$ (101.4 ± 83.0 vs. 101.4 ± 78.8 , respectively, $p = 1$). Further statistical and PK analyses were conducted using $eCONC_{Plasma}$ derived by this linear regression method.

Bland-Altman analysis revealed that the geometric mean ampicillin concentration ratios between $CONC_{DBS}$ and $CONC_{Plasma}$, as well as $eCONC_{Plasma}$ and $CONC_{Plasma}$, were 0.56 (95% confidence interval [CI], 0.31 to 0.89) and 0.88 (95% CI, 0.31 to 1.58), respectively. The ratio of $eCONC_{Plasma} / CONC_{Plasma}$ was significantly higher than that of $CONC_{DBS} /$

CONC_{Plasma} (Wilcoxon signed-rank test, $n=29$, $p<0.001$). One CONC_{DBS} fell outside the 95% CI (Figure 2A). All eCONC_{Plasma} were within the 95% limits of agreement (Figure 2B).

Population PK Analysis

A total of 35 plasma and 30 DBS samples from 18 subjects were included in the pop-PK analysis. All CONC_{Plasma} and CONC_{DBS} data were above the LLOQ. The median (range) time after last dose of PK sampling, CONC_{Plasma}, and CONC_{DBS} data were 5.7 (0.3–14.3) hours, 86 (7–293) $\mu\text{g/mL}$, and 47 (4–158) mcg/mL , respectively. The number of CONC_{Plasma} measured from the beginning of infusion to 1.0 hour was 7 (20%), 1.1–2.0 hours was 3 (9%), 2.1–5.0 hours was 7 (20%), 5.1–8.0 hours was 5 (14%), and >8 hours was 13 (37%).

The final pop-PK model, derived from CONC_{Plasma} using FOCE-I with combination residual error, was applied to the CONC_{DBS} and eCONC_{Plasma} matrices (Table 3). The median bias (interquartile range) of the CONC_{DBS} was –44% (–51 to –34), which improved to –11% (–56 to 14) when CONC_{DBS} was transformed to eCONC_{Plasma}. Using the final pop-PK model, the median bias of clearance and individual predicted concentrations improved by 62% and 35%, respectively, when transformed DBS concentrations were used (Table 4).

Discussion

In our previously published study, a broad age spectrum of neonates was incorporated to enable the construct of a pop-PK model, reflecting developmental changes, that was subsequently used to simplify ampicillin dosing regimens.⁵ Here, a subset of the data with paired DBS-plasma samples was used for comparative analyses using PK modeling. While correlation analysis demonstrated strong association between CONC_{Plasma} and CONC_{DBS}, the bias exceeded >15%, demonstrating that bias was present.^{14–16} Bias between plasma and DBS concentrations was expected based on *in vitro* data suggesting lack of ampicillin penetration into red blood cells. A similar phenomenon was observed with *in vivo* data as evidenced by the ratio of DBS to plasma concentrations. The differences between DBS (whole blood) and plasma could have been explained by evaluating the role of hematocrit in DBS concentrations. Nevertheless, a hematocrit correction was not performed because 1) in the context of an opportunistic study, there was no access to the closest hematocrit measured prior to PK sample collection; 2) of avoidance in evaluating the effect of individual differences in red blood cell penetration in these analyses; and 3) of the goal to explore a practical approach for DBS concentration management in PK analyses. Therefore, the focus was on the use of statistical models to transform DBS concentration data to plasma. Furthermore, the same observed bias is retained when either the pop-PK or statistical approach is used.

Based on eCONC_{Plasma}, which was transformed to CONC_{DBS} using linear regression, the ampicillin clearance and exposure improved to values similar to those obtained from observed CONC_{Plasma}. Simple linear regression has been used in other clinical studies to transform DBS into estimated plasma concentrations.^{13,17} Alternatively, the ratio

transformation method has been evaluated for antiepileptic drugs in children.¹⁸ With ampicillin displaying a wide therapeutic index, fluctuations of drug concentrations within a reasonable margin should not affect efficacy and safety, which implies that the accuracy margin to use DBS (albeit transformed and not directly) to predict ampicillin exposure may be less restrictive. As a result, the use of DBS samples in this opportunistic study provided the *in vivo* relationship between whole blood and plasma concentrations of ampicillin and, more importantly, a practical approach for PK sampling to predict drug exposure. In addition, since hematocrit changes with age and was not incorporated in the linear regression, the transformation method to estimate $eCONC_{Plasma}$ is only applicable to neonates.

The DBS sampling approach has been studied for both research purposes (as in this study) and clinical use, including therapeutic drug monitoring and newborn screening for congenital metabolic diseases. Various antibiotics have been evaluated using DBS technology in pediatric research.^{11–13,19,20} In clinical practice, the use of DBS as an alternative to plasma concentrations has been applied to drugs with a narrow therapeutic index that require drug monitoring to individualize dosages, which is a critical aspect in pediatric care due to PK variations.^{18,21,22} Clinical decisions can be made based on DBS concentrations in different patient care settings, including the outpatient communities where patient convenience may also be enhanced. Furthermore, the DBS strategy may prove to be cost-effective and invaluable in regions and countries with limited resources, as previously demonstrated by viral-load-informed differentiated care in patients with human immunodeficiency virus.²³

Limitations

This study had several limitations. First, the small sample size and sparse sampling of both the $CONC_{Plasma}$ and $CONC_{DBS}$ data prevented the application of our published model, due to over-parameterization and large relative standard error. Second, the $CONC_{Plasma}$ and $CONC_{DBS}$ were assayed at different laboratories, which may have contributed to the variability. Finally, the DBS sampling technique requires uniform spot coverage on the card, rapid spot drying, and storage in low humidity. In the absence of these conditions, variability in $CONC_{DBS}$ may have been introduced.

More research is needed to explore and validate the DBS microsampling technique in PK studies for neonates and pediatrics, especially those who are critically ill, to augment the overall understanding of the altered pharmacokinetic properties resulting from maturation and severity of illness.²⁴ Furthermore, this sampling strategy should be studied for currently marketed and new drugs, especially those with a narrow therapeutic index if intended for integration into the clinical setting.

Conclusions

Ampicillin DBS concentrations were successfully used to estimate plasma concentrations in this pop-PK study in neonates. The practicality, convenience, and enhanced parental acceptance of DBS sampling, as compared with traditional plasma, makes this approach attractive for research and clinical use.

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References

1. Le, J., Bradley, JS. Pharmacodynamic considerations and special populations: pediatrics. In: Rotschafer, JC, Andes, DR., Rodvold, KA., editors. *Antibiotic Pharmacodynamics*. New York: Springer; 2016. p. 561-597.
2. Cohen-Wolkowicz M, Benjamin DK Jr, Ross A, et al. Population pharmacokinetics of piperacillin using scavenged samples from preterm infants. *Ther Drug Monit*. 2012; 34:312-319. [PubMed: 22569355]
3. Cohen-Wolkowicz M, Ouellet D, Smith PB, et al. Population pharmacokinetics of metronidazole evaluated using scavenged samples from preterm infants. *Antimicrob Agents Chemother*. 2012; 56:1828-1837. [PubMed: 22252819]
4. Le J, Bradley JS, Murray W, et al. Improved vancomycin dosing in children using area under the curve exposure. *Pediatr Infect Dis J*. 2013; 32:e155-e163. [PubMed: 23340565]
5. Tremoulet A, Le J, Poindexter B, et al. Characterization of the population pharmacokinetics of ampicillin in neonates using an opportunistic study design. *Antimicrob Agents Chemother*. 2014; 58:3013-3020. [PubMed: 24614374]
6. Wade KC, Wu D, Kaufman DA, et al. Population pharmacokinetics of fluconazole in young infants. *Antimicrob Agents Chemother*. 2008; 52:4043-4049. [PubMed: 18809946]

7. Evans C, Arnold M, Bryan P, et al. Implementing dried blood spot sampling for clinical pharmacokinetic determinations: considerations from the IQ Consortium Microsampling Working Group. *AAPS J.* 2015; 17:292–300. [PubMed: 25488054]
8. McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography.* 2007; 44:899–925. [PubMed: 18232218]
9. De Jesus VR, Zhang XK, Keutzer J, et al. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. *Clin Chem.* 2009; 55:158–164. [PubMed: 18988750]
10. Centers for Disease Control and Prevention (CDC). [Accessed July 21, 2017] Laboratory quality assurance and standardization programs. CDC web site: <https://www.cdc.gov/labstandards/overview.html>
11. Cohen-Wolkowicz M, Sampson M, Bloom BT, et al. Determining population and developmental pharmacokinetics of metronidazole using plasma and dried blood spot samples from premature infants. *Pediatr Infect Dis J.* 2013; 32:956–961. [PubMed: 23587979]
12. Suyagh M, Collier PS, Millership JS, et al. Metronidazole population pharmacokinetics in preterm neonates using dried blood-spot sampling. *Pediatrics.* 2011; 127:e367–e374. [PubMed: 21220396]
13. Cohen-Wolkowicz M, Watt KM, Zhou C, et al. Developmental pharmacokinetics of piperacillin and tazobactam using plasma and dried blood spots from infants. *Antimicrob Agents Chemother.* 2014; 58:2856–2865. [PubMed: 24614369]
14. Parsons TL, Marzinke MA, Hoang T, et al. Quantification of rifapentine, a potent antituberculosis drug, from dried blood spot samples using liquid chromatographic-tandem mass spectrometric analysis. *Antimicrob Agents Chemother.* 2014; 58:6747–6757. [PubMed: 25182637]
15. David OJ, Johnston A. Limited sampling strategies for estimating cyclosporin area under the concentration-time curve: review of current algorithms. *Ther Drug Monit.* 2001; 23:100–114. [PubMed: 11294509]
16. Ting LS, Villeneuve E, Ensom MH. Beyond cyclosporine: a systematic review of limited sampling strategies for other immunosuppressants. *Ther Drug Monit.* 2006; 28:419–430. [PubMed: 16778729]
17. Vu DH, Bolhuis MS, Koster RA, et al. Dried blood spot analysis for therapeutic drug monitoring of linezolid in patients with multidrug-resistant tuberculosis. *Antimicrob Agents Chemother.* 2012; 56:5758–5763. [PubMed: 22926568]
18. Linder C, Wide K, Walander M, et al. Comparison between dried blood spot and plasma sampling for therapeutic drug monitoring of antiepileptic drugs in children with epilepsy: a step towards home sampling. *Clin Biochem.* 2017; 50:418–424. [PubMed: 28027888]
19. Carlier M, Stove V, Wallis SC, et al. Assays for therapeutic drug monitoring of beta-lactam antibiotics: a structured review. *Int J Antimicrob Agents.* 2015; 46:367–375. [PubMed: 26271599]
20. Gonzalez D, Palazzi DL, Bhattacharya-Mithal L, et al. Solithromycin pharmacokinetics in plasma and dried blood spots and safety in adolescents. *Antimicrob Agents Chemother.* 2016; 60:2572–2576. [PubMed: 26883693]
21. Bruschetti M, Barco S, Romantsik O, et al. DBS-LC-MS/MS assay for caffeine: validation and neonatal application. *Bioanalysis.* 2016; 8:1893–1902. [PubMed: 27532249]
22. Dickerson JA, Sinkey M, Jacot K, et al. Tacrolimus and sirolimus in capillary dried blood spots allows for remote monitoring. *Pediatr Transplant.* 2015; 19:101–106. [PubMed: 25414084]
23. Working Group on Modelling of Antiretroviral Therapy Monitoring Strategies in Sub-Saharan Africa. Phillips A, Shroufi A, et al. Sustainable HIV treatment in Africa through viral-load-informed differentiated care. *Nature.* 2015; 528:S68–S76. [PubMed: 26633768]
24. Dorofaeff T, Bandini RM, Lipman J, et al. Uncertainty in antibiotic dosing in critically ill neonate and pediatric patients: can microsampling provide the answers? *Clin Ther.* 2016; 38:1961–1975. [PubMed: 27544661]

Figure 1. Relationship between plasma and DBS concentrations

Relationship between plasma and DBS concentrations: A) concentration (log scale) vs. time; solid and dashed lines represent plasma and dried blood spot linear trends, respectively; and B) linear regression (solid line represents mean $[CONC_{DBS}] = 3.223 + 0.51 * [CONC_{plasma}]$ and dashed line 95% CI; $R^2=0.902$ [slope 0.51 with 95% CI = 0.444 – 0.577])

Abbreviations: CI = confidence interval; DBS = dried blood spot

Figure 2. Bland-Altman plots

Bland-Altman plots of A) plasma concentrations and DBS (the solid black line denotes the mean ratio of 0.58 and dashed lines 95% CI) and B) estimated plasma (derived from dried blood spot [DBS]) and plasma concentrations (the solid black line denotes the mean ratio of 0.79 and dashed lines 95% CI; estimated plasma concentrations were calculated using the equation $[CONC_{DBS} - 3.223] / 0.51$ that was derived from simple linear regression).

Abbreviations: CI = confidence interval; DBS = dried blood spot

Table 1
Demographic characteristics (N=18)

Birth weight (grams)	
Median (range)	3120 (930–4110)
Gestational age (weeks)	
Median (range)	37.3 (27.0–41.0)
Postnatal age (days)	
Median (min, max)	8 (1–26)
Male sex	
	9 (50%)
Ethnicity	
Hispanic or Latino	3 (17%)
Not Hispanic or Latino	12 (67%)
Not reported	3 (17%)
Race	
Black	1 (6%)
White	15 (83%)
Native Hawaiian	1 (6%)
Not reported	1 (6%)
Serum creatinine (mg/dL)	
Median (range)	0.50 (0.30–1.10)

Table 2
Transformation methods to estimate plasma concentrations using DBS concentrations

Method	Equation	Estimated Plasma Concentrations ²	p value ³
Ratio	$\text{CONC}_{\text{DBS}} / 0.58$ ¹	94.7 ± 73.0	0.2
Linear regression	$(\text{CONC}_{\text{DBS}} - 3.223) / 0.51$	101.4 ± 83.0	1
Log-linear regression	$(\text{Log} [\text{CONC}_{\text{DBS}}] + 0.098) / 0.917$	1.8 ± 0.5	1

Abbreviations: DBS = dried blood spot

¹ Derived using the mean (± 0.15) of the DBS over plasma concentration ratio.

² Numbers represent mean $\pm$ standard deviation.

³ Compared with the mean measured plasma concentrations ($\text{CONC}_{\text{Plasma}}$ that was 101.4 ± 78.8 for ratio and linear regression and 1.8 ± 0.5 for log-linear regression methods).

Table 3

Final population-based pharmacokinetic model parameters¹

Parameter	Symbol	Measured plasma concentration ²			Dried blood spot			Estimated plasma concentration ³		
		Point estimate	%RSE		Point estimate	%RSE		Point estimate	%RSE	
CL	θ_{CL}	3.42	9.44		6.10	12.93		4.25	17.76	
V	θ_V	28.2	5.18		60.80	9.47		27.50	7.05	
CL, SCR	θ_{SCR}	-1.13	-15.13		-1.05	-4.94		-1.32	-9.09	
CL inter-individual variability (CV%)	ω^2_{CL}	33.0	44.0		34.35	68.31		55.32	48.69	
Residual variability (CV%)	$\sigma^2_{Proportional}$	9.86	69.44		17.38	57.28		10.82	71.45	
Residual variability	$\sigma_{Additive}$	165	44.79		81	41.69		377	55.44	

Abbreviations: CL = clearance (L/hour); CV% = coefficient of variance (%); %RSE = percent relative standard error; SCR = serum creatinine (mg/dL); V = volume (L); WTKG = weight (kg)

¹The CV% and %RSE were calculated using: $100 \times SD / \text{mean}$ and $100 \times SE / \text{estimate}$, respectively.

²Final model derived using measured plasma concentrations was $V = \theta_V * (WTKG/70)^1$ and $CL = \theta_{CL} * (WTKG/70)^{0.75} * ([1 + \theta_{SCR}] * [SCR - 0.50])$. The ETA shrinkage value for CL was 9%, while the EPS shrinkage value for CL was 23%.

³Estimated plasma concentrations were calculated using the linear regression transformation method $([CONCDBS] - 3.223) / 0.51$.

Table 4
Individual empiric Bayesian post-hoc parameter estimates based on sampling strategy¹

Sampling Strategy	Clearance (L/hour/kg)	Volume (L/kg)	Half-life (hour)	Individual Predicted Concentration (mcg/mL)
Measured plasma	0.10 (0.07 to 0.14)	0.40 (0.40 to 0.40)	2.75 (1.96 to 3.68)	87 (27 to 163)
DBS	0.19 (0.13 to 0.24)	0.87 (0.87 to 0.87)	3.13 (2.48 to 4.55)	46 (26 to 90)
Bias (%) ²	70 (56 to 95)	115 (115 to 115)	27 (11 to 38)	−43 (−50 to −20)
Estimated plasma ³	0.11 (0.07 to 0.19)	0.39 (0.39 to 0.39)	2.52 (1.42 to 3.68)	79 (30 to 169)
Bias (%) ²	8 (−11 to 50)	−3 (−3 to −3)	−10 (−35 to 9)	−8 (−34 to 11)

Abbreviations: DBS = dried blood spot

¹Numbers expressed are median (interquartile range).

²Bias was calculated by $100\% * (Y_{DBS} [or ePlasma] - Y_{Plasma}) / Y_{Plasma}$.

³Estimated plasma concentrations were calculated using the linear regression transformation method: $(CONC_{DBS} - 3.223) / 0.51$.